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jc803 U.S. PTO



07/21/00

Date: July 21, 2000

Docket No.: 2750-991P

jc803 U.S. PTO
09/621630



07/21/00

BOX PATENT APPLICATION

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

As authorized by the inventor(s), transmitted herewith for filing is a patent application applied for on behalf of the inventor(s) according to the provisions of 37 C.F.R. § 1.41(c), which claims priority under 35 U.S.C. § 119(e) of Provisional Application No. 60/144,814 filed on July 21, 1999

Inventor(s): Nickolai ALEXANDROV, Maxim TROUKHAN

For: SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY

Enclosed are:

☒ A specification consisting of a Description (844 pages), Table 1 (25 pages), Claims (5 pages), schematic (1 page), Abstract (1 page) totaling eight hundred seventy six (876) pages

☐ () sheet(s) of formal drawings

☐ Certified copy of Priority Document(s)

☒ Executed Declaration in accordance with 37 C.F.R. § 1.64 will follow

☒ A statement to establish small entity status under 37 C.F.R. § 1.9 and 37 C.F.R. § 1.27

- ☐ Preliminary Amendment
- ☒ Information Sheet
- ☐ Information Disclosure Statement, PTO-1449 and reference(s)
- ☐ Amend the specification by inserting before the first line the sentence:

--This application claims priority on provisional Application No. _____ filed on _____, the entire contents of which are hereby incorporated by reference.--

- ☒ Other: Power of Attorney regarding Small Entity Statement, ATCC Deposit receipts PTA-595, PTA-1161, PTA-1411, CD containing Specification

The filing fee has been calculated as shown below:

			LARGE ENTITY		SMALL ENTITY	
BASIC FEE			\$690.00		\$345.00	
	NUMBER FILED	NUMBER EXTRA	RATE	FEE	RATE	FEE
TOTAL CLAIMS	50- 20 =	30	X 18 =	\$0.00	x 9 =	270
INDEPENDENT CLAIMS	5- 3 =	2	x 78 =	\$0.00	x 39 =	78
MULTIPLE DEPENDENT <input type="checkbox"/> CLAIMS PRESENTED			+ \$260.00		+ \$130.00	
TOTAL			\$0.00		\$693.00	

- ☒ The application transmitted herewith is filed in accordance with 37 C.F.R. § 1.41(c). The undersigned has been authorized by the inventor(s) to file the present application. The original duly executed declaration together with the surcharge will be forwarded in due course.
- ☒ A check in the amount of \$693.00 to cover the filing fee is enclosed.

☐ Please charge Deposit Account No. 02-2448 in the amount of \$0.00. A triplicate copy of this transmittal form is enclosed.

☒ Please send correspondence to:

BIRCH, STEWART, KOLASCH & BIRCH, LLP or Customer No. 2292
P.O. Box 747
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Telephone: (703) 205-8000

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 
Raymond C. Stewart, #21,066

RCS/JRK
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Attachments

STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) & 1.27(c)) - SMALL BUSINESS CONCERN

Docket Number: 2750-991P

Applicant, Patentee, or Identifier: N. ALEXANDROV et al.

Application or Patent No.: NEW Patent Application

Filed or Issued: July 21, 2000

Title: SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES
ENCODED THEREBY

I hereby state that I am

- ☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN CERES, INC.

ADDRESS OF SMALL BUSINESS CONCERN 3007 Malibu Canyon Road Malibu, CA 90265

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 37 CFR Part 121 for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

- ☒ the specification filed herewith with title as listed above.
☐ the application identified above.
☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern, or organization having rights in the invention must file separate statements as to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization having any rights in the invention is listed below:

- ☒ no such person, concern, or organization exists.
☐ each such person, concern, or organization is listed below.

Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is not longer appropriate. (37 CFR 1.28(b))

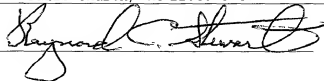
NAME OF PERSON SIGNING Raymond C. Stewart (Reg. No. 21,066)

TITLE IN ORGANIZATION OF PERSON SIGNING Legal Representative of CERES, INC.

ADDRESS OF PERSON SIGNING Birch, Stewart, Kolasch and Birch, LLP.

P.O. Box 747 Falls Church, VA 22040-0747

SIGNATURE



DATE July 21, 2000

**SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING
POLYPEPTIDES ENCODED THEREBY**

This application claims priority under 35 USC §119(e), §119(a-d) and §120 of the following applications, the entire contents of which are hereby incorporated by reference:

Country	Filing Date	Attorney No.	Client No.	Application No.
United States	07/21/99	2750-0503P	00066.001	60/144,814

FIELD OF THE INVENTION

The present invention relates to isolated polynucleotides that represent a complete gene, or a fragment thereof, that is expressed. In addition, the present invention relates to the polypeptide or protein corresponding to the coding sequence of these polynucleotides. The present invention also relates to isolated polynucleotides that represent regulatory regions of genes. The present invention also relates to isolated polynucleotides that represent untranslated regions of genes. The present invention further relates to the use of these isolated polynucleotides and polypeptides and proteins.

DESCRIPTION OF THE RELATED ART

Efforts to map and sequence the genome of a number of organisms are in progress; a few complete genome sequences, for example those of *E. coli* and *Saccharomyces cerevisiae* are known (Blattner et al., *Science* 277:1453 (1997); Goffeau et al., *Science* 274:546 (1996)). The complete genome of a multicellular organism, *C. elegans*, has also been sequenced (See, the *C. elegans* Sequencing Consortium, *Science* 282:2012 (1998)). To date, no complete genome of a plant has been sequenced, nor has a complete cDNA complement of any plant been sequenced.

SUMMARY OF THE INVENTION

The present invention comprises polynucleotides, such as complete cDNA sequences and/or sequences of genomic DNA encompassing complete genes, fragments of genes, and/or regulatory elements of genes and/or regions with other functions and/or intergenic regions, hereinafter collectively referred to as Sequence-Determined DNA Fragments (SDFs), from different plant species, particularly corn, wheat, soybean, rice and *Arabidopsis thaliana*, and other plants and or mutants, variants, fragments or fusions of said SDFs and polypeptides or proteins derived therefrom. In some instances, the SDFs span the entirety of a protein-coding segment. In some instances, the entirety of an mRNA is represented. Other objects of the invention that are also represented by SDFs of the invention are control sequences, such as, but

not limited to, promoters. Complements of any sequence of the invention are also considered part of the invention.

Other objects of the invention are polynucleotides comprising exon sequences, polynucleotides comprising intron sequences, polynucleotides comprising introns together with exons, intron/exon junction sequences, 5' untranslated sequences, and 3' untranslated sequences of the SDFs of the present invention. Polynucleotides representing the joinder of any exons described herein, in any arrangement, for example, to produce a sequence encoding any desirable amino acid sequence are within the scope of the invention.

The present invention also resides in probes useful for isolating and identifying nucleic acids that hybridize to an SDF of the invention. The probes can be of any length, but more typically are 12-2000 nucleotides in length; more typically, 15 to 200 nucleotides long; even more typically, 18 to 100 nucleotides long.

Yet another object of the invention is a method of isolating and/or identifying nucleic acids using the following steps:

- (a) contacting a probe of the instant invention with a polynucleotide sample under conditions that permit hybridization and formation of a polynucleotide duplex; and
- (b) detecting and/or isolating the duplex of step (a).

The conditions for hybridization can be from low to moderate to high stringency conditions. The sample can include a polynucleotide having a sequence unique in a plant genome. Probes and methods of the invention are useful, for example, without limitation, for mapping of genetic traits and/or for positional cloning of a desired fragment of genomic DNA.

Probes and methods of the invention can also be used for detecting alternatively spliced messages within a species. Probes and methods of the invention can further be used to detect or isolate related genes in other plant species using genomic DNA (gDNA) and/or cDNA libraries. In some instances, especially when longer probes and low to moderate stringency hybridization conditions are used; the probe will hybridize to a plurality of cDNA and/or gDNA sequences of a plant. This approach is useful for isolating representatives of gene families which are identifiable by possession of a common functional domain in the gene product or which have common cis-acting regulatory sequences. This approach is also useful for identifying orthologous genes from other organisms.

The present invention also resides in constructs for modulating the expression of the genes comprised of all or a fragment of an SDF. The constructs comprise all or a fragment of the expressed SDF, or of a complementary sequence. Examples of constructs include ribozymes comprising RNA encoded by an SDF or by a sequence complementary thereto,

antisense constructs, constructs comprising coding regions or parts thereof, constructs comprising promoters, introns, untranslated regions, scaffold attachment regions, methylating regions, enhancing or reducing regions, DNA and chromatin conformation modifying sequences, etc. Such constructs can be constructed using viral, plasmid, bacterial artificial chromosomes (BACs), plasmid artificial chromosomes (PACs), autonomous plant plasmids, plant artificial chromosomes or other types of vectors and exist in the plant as autonomous replicating sequences or as DNA integrated into the genome. When inserted into a host cell the construct is, preferably, functionally integrated with, or operatively linked to, a heterologous polynucleotide. For instance, a coding region from an SDF might be operably linked to a promoter that is functional in a plant.

The present invention also resides in host cells, including bacterial or yeast cells or plant cells, and plants that harbor constructs such as described above. Another aspect of the invention relates to methods for modulating expression of specific genes in plants by expression of the coding sequence of the constructs, by regulation of expression of one or more endogenous genes in a plant or by suppression of expression of the polynucleotides of the invention in a plant. Methods of modulation of gene expression include without limitation (1) inserting into a host cell additional copies of a polynucleotide comprising a coding sequence; (2) modulating an endogenous promoter in a host cell; (3) inserting antisense or ribozyme constructs into a host cell and (4) inserting into a host cell a polynucleotide comprising a sequence encoding a variant , fragment, or fusion of the native polypeptides of the instant invention.

BRIEF DESCRIPTION OF THE TABLES

In TABLE 1, the format of the data is as follows:

In Table 1, sequence data are presented in the form of annotation of a reference sequence. The format is shown below. The reference sequence is shown at the top of the annotation file as a 7 digit sequence number preceded by ">" (e.g. >5019261). The sequence identifier is a "gi" number that identifies a specific DNA sequence in the publically accessible BLAST Databases on the NCBI FTP web site (accessible at ncbi.nlm.gov/blast). In particular, the "nt.Z" nucleotide sequence data base at the NCBI FTP site utilizes the "gi" identifiers to assign by NCBI a unique identifier for each sequence in the databases, thereby providing a non-redundant database for sequences from various data bases, including GenBank, EMBL, DDBJ (DNA Database of Japan) and PDB (Brookhaven Protein Data Bank). Thus, the line in TABLE 1 beginning with sequence number identifies the unique "gi" identifier followed by the corresponding GenBank (gb) accession number and locus. The

reference sequence number is followed on the next line by data regarding the length of the sequence (len") and the number of exons found in the sequence by the analysis program (nex").

- 5 The annotation data are presented in columns; the leftmost column identifies the position of the putative exon in the gene as initial (init"), internal (intr") or terminal (term"). Genes considered composed of a single exon are denoted snl". The next column describes the position in the nucleotide sequence beginning the exon (start") and the next column describes the position in the nucleotide sequence ending the exon (stop"). The direction of the gene is indicated in the next column, +" indicating 5' - 3' in the direction presented in
- 10 the database, - indicating the opposite orientation. The gene number" is given in the final column. Exons having the same gene number are grouped in the order shown to create the relevant coding sequence.

>5019261 ← This is the gi number of the public sequence

len = 97208 nex = 121

↑

↑

Length

Number exons

5 of public sequence

	Exon	Start	Stop	Direction	Gene
	Type				Number
10	↓	↓	↓	↓	↓
	Sngl	602	778	+	0
	Sngl	990	1316	+	1
	Sngl	2356	2691	+	2
	Sngl	4634	4733	+	3
15	Sngl	4973	5092	+	4
	Sngl	5746	5874	+	5
	Init	8119	8798	+	6
	Term	9284	9518	+	6
	Init	10827	11150	+	7
20	Term	11294	11335	+	7
	Sngl	12655	12825	+	8
	Sngl	13303	13596	+	9
	Sngl	18654	18782	+	10
	Sngl	19880	20086	+	11
25	Init	21476	21539	+	12
	Intr	21647	21802	+	12
	Term	23488	23567	=	12
	Init	25035	25133	+	13
	Intr	25466	25589	+	13
30	Intr	25677	25786	+	13
	Intr	25899	25962	+	13
	Intr	26045	26109	+	13
	Intr	26188	26253	+	13
	Term	26350	26448	+	13
35	Sngl	27671	27793	+	14
	Sngl	29126	29299	+	15
	Sngl	30266	30364	+	16
	Sngl	31717	31929	+	17
	Sngl	32102	32209	+	18
40	Sngl	32450	32548	+	19

	Sngl	32634	32726	+	20
	Init	35603	35743	+	21
	Term	35829	36185	+	21
	Init	36954	37098	+	22
5	Term	38100	38158	+	22
	Init	39635	39944	+	23
	Intr	40242	40372	+	23
	Intr	40462	40695	+	23
	Intr	40815	41070	+	23
10	Intr	41176	41255	+	23
	Intr	42212	42419	+	23
	Intr	42940	43070	+	23
	Intr	43177	43410	+	23
	Intr	43580	43835	+	23
15	Intr	46672	46715	+	23
	Intr	48334	48532	+	23

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to (I) polynucleotides and methods of use thereof, such as

IA. Probes, Primers and Substrates;

IB. Methods of Detection and Isolation;

B.1. Hybridization;

B.2. Methods of Mapping;

B.3. Southern Blotting;

B.4. Isolating cDNA from Related Organisms;

B.5. Isolating and/or Identifying Orthologous Genes

IC. Methods of Inhibiting Gene Expression

C.1. Antisense

C.2. Ribozyme Constructs;

C.3. Chimeraplasts;

C.4. Co-Suppression;

C.5. Transcriptional Silencing

C.6. Other Methods to Inhibit Gene Expression

ID. Methods of Functional Analysis;

IE. Promoter Sequences and Their Use;

IF. UTRs and/or Intron Sequences and Their Use; and

IG. Coding Sequences and Their Use.

The invention also relates to (II) polypeptides and proteins and methods of use thereof, such as IIA. Native Polypeptides and Proteins

5

A.1 Antibodies

A.2 In Vitro Applications

IIB. Polypeptide Variants, Fragments and Fusions

B.1 Variants

B.2 Fragments

10

B.3 Fusions

The invention also includes (III) methods of modulating polypeptide production, such as

IIIA. Suppression

A.1 Antisense

15

A.2 Ribozymes

A.3 Co-suppression

A.4 Insertion of Sequences into the Gene to be Modulated

A.5 Promoter Modulation

A.6 Expression of Genes containing Dominant-Negative Mutations

20

IIIB. Enhanced Expression

B.1 Insertion of an Exogenous Gene

B.2 Promoter Modulation

The invention further concerns (IV) gene constructs and vector construction, such as

25

IVA. Coding Sequences

IVB. Promoters

IVC. Signal Peptides

The invention still further relates to

30

V Transformation Techniques

Definitions

Allelic variant An "allelic variant" is an alternative form of the same SDF, which resides at the same chromosomal locus in the organism. Allelic variations can occur in any portion of the gene sequence, including regulatory regions. Allelic variants can arise by normal genetic variation in a population. Allelic variants can also be produced by genetic engineering methods. An allelic variant can be one that is found in a naturally occurring plant, including a cultivar or ecotype. An allelic variant may or may not give rise to a phenotypic change, and may or may not be expressed. An allele can result in a detectable change in the phenotype of the trait represented by the locus. A phenotypically silent allele can give rise to a product.

Alternatively spliced messages Within the context of the current invention, "alternatively spliced messages" refers to mature mRNAs originating from a single gene with variations in the number and/or identity of exons, introns and/or intron-exon junctions.

Chimeric The term "chimeric" is used to describe genes, as defined supra, or constructs wherein at least two of the elements of the gene or construct, such as the promoter and the coding sequence and/or other regulatory sequences and/or filler sequences and/or complements thereof, are heterologous to each other.

Constitutive Promoter: Promoters referred to herein as "constitutive promoters" actively promote transcription under most, but not necessarily all, environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcript initiation region and the 1' or 2' promoter derived from T-DNA of *Agrobacterium tumefaciens*, and other transcription initiation regions from various plant genes, such as the maize ubiquitin-1 promoter, known to those of skill.

Coordinately Expressed: The term "coordinately expressed," as used in the current invention, refers to genes that are expressed at the same or a similar time and/or stage and/or under the same or similar environmental conditions.

Domain: Domains are fingerprints or signatures that can be used to characterize protein families and/or parts of proteins. Such fingerprints or signatures can comprise conserved (1) primary sequence, (2) secondary structure, and/or (3) three-dimensional conformation. Generally, each domain has been associated with either a family of proteins or motifs. Typically, these families and/or motifs have been correlated with specific *in-vitro* and/or *in-vivo* activities. A domain can be any length, including the entirety of the sequence of a protein. Detailed descriptions of the domains, associated families and motifs, and correlated activities of the polypeptides of the instant invention are described below. Usually, the polypeptides with designated domain(s) can exhibit at least one activity that is exhibited by any polypeptide that comprises the same domain(s).

Endogenous The term "endogenous," within the context of the current invention refers to any polynucleotide, polypeptide or protein sequence which is a natural part of a cell or organisms regenerated from said cell.

Exogenous "Exogenous," as referred to within, is any polynucleotide, polypeptide or protein sequence, whether chimeric or not, that is initially or subsequently introduced into the genome of an individual host cell or the organism regenerated from said host cell by any means other than by a sexual cross. Examples of means by which this can be accomplished are described below, and include *Agrobacterium*-mediated transformation (of dicots - e.g. Salomon et al. *EMBO J.* 3:141 (1984); Herrera-Estrella et al. *EMBO J.* 2:987 (1983); of monocots, representative papers are those by Escudero et al., *Plant J.* 10:355 (1996), Ishida et al., *Nature Biotechnology* 14:745 (1996), May et al., *Bio/Technology* 13:486 (1995)), biolistic methods (Armaleo et al., *Current Genetics* 17:97 (1990)), electroporation, *in planta* techniques, and the like. Such a plant containing the exogenous nucleic acid is referred to here as a T₀ for the primary transgenic plant and T₁ for the first generation. The term "exogenous" as used herein is also intended to encompass inserting a naturally found element into a non-naturally found location.

Filler sequence: As used herein, "filler sequence" refers to any nucleotide sequence that is inserted into DNA construct to evoke a particular spacing between particular components such as a promoter and a coding region and may provide an additional attribute such as a restriction enzyme site.

Gene: The term "gene," as used in the context of the current invention, encompasses all regulatory and coding sequence contiguously associated with a single hereditary unit with a genetic function (see SCHEMATIC 1). Genes can include non-coding sequences that
5 modulate the genetic function that include, but are not limited to, those that specify polyadenylation, transcriptional regulation, DNA conformation, chromatin conformation, extent and position of base methylation and binding sites of proteins that control all of these. Genes comprised of "exons" (coding sequences), which may be interrupted by "introns" (non-coding sequences), encode proteins. A gene's genetic function may require only RNA
10 expression or protein production, or may only require binding of proteins and/or nucleic acids without associated expression. In certain cases, genes adjacent to one another may share sequence in such a way that one gene will overlap the other. A gene can be found within the genome of an organism, artificial chromosome, plasmid, vector, etc., or as a separate isolated entity.

Gene Family: "Gene family" is used in the current invention to describe a group of functionally related genes, each of which encodes a separate protein.

Heterologous sequences: "Heterologous sequences" are those that are not operatively
20 linked or are not contiguous to each other in nature. For example, a promoter from corn is considered heterologous to an *Arabidopsis* coding region sequence. Also, a promoter from a gene encoding a growth factor from corn is considered heterologous to a sequence encoding the corn receptor for the growth factor. Regulatory element sequences, such as UTRs or 3' end termination sequences that do not originate in nature from the same gene as the coding sequence
25 originates from, are considered heterologous to said coding sequence. Elements operatively linked in nature and contiguous to each other are not heterologous to each other. On the other hand, these same elements remain operatively linked but become heterologous if other filler sequence is placed between them. Thus, the promoter and coding sequences of a corn gene expressing an amino acid transporter are not heterologous to each other, but the promoter and
30 coding sequence of a corn gene operatively linked in a novel manner are heterologous.

Homologous gene In the current invention, "homologous gene" refers to a gene that shares sequence similarity with the gene of interest. This similarity may be in only a fragment of the sequence and often represents a functional domain such as, examples including without

limitation a DNA binding domain, a domain with tyrosine kinase activity, or the like. The functional activities of homologous genes are not necessarily the same.

Inducible Promoter

An "inducible promoter" in the context of the current invention refers to a promoter which is regulated under certain conditions, such as light, chemical concentration, protein concentration, conditions in an organism, cell, or organelle, etc. A typical example of an inducible promoter, which can be utilized with the polynucleotides of the present invention, is PARSK1, the promoter from the *Arabidopsis* gene encoding a serine-threonine kinase enzyme, and which promoter is induced by dehydration, abscissic acid and sodium chloride (Wang and Goodman, *Plant J.* 8:37 (1995)) Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light.

Intergenic region

"Intergenic region," as used in the current invention, refers to nucleotide sequence occurring in the genome that separates adjacent genes.

Mutant gene

In the current invention, "mutant" refers to a heritable change in DNA sequence at a specific location. Mutants of the current invention may or may not have an associated identifiable function when the mutant gene is transcribed.

Orthologous Gene

In the current invention "orthologous gene" refers to a second gene that encodes a gene product that performs a similar function as the product of a first gene. The orthologous gene may also have a degree of sequence similarity to the first gene. The orthologous gene may encode a polypeptide that exhibits a degree of sequence similarity to a polypeptide corresponding to a first gene. The sequence similarity can be found within a functional domain or along the entire length of the coding sequence of the genes and/or their corresponding polypeptides.

Percentage of sequence identity

"Percentage of sequence identity," as used herein, is determined by comparing two optimally aligned sequences over a comparison window, where the fragment of the polynucleotide or amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences.

- The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Add. APL. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (USA)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, PASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection. Given that two sequences have been identified for comparison, GAP and BESTFIT are preferably employed to determine their optimal alignment. Typically, the default values of 5.00 for gap weight and 0.30 for gap weight length are used. The term "substantial sequence identity" between polynucleotide or polypeptide sequences refers to polynucleotide or polypeptide comprising a sequence that has at least 80% sequence identity, preferably at least 85%, more preferably at least 90% and most preferably at least 95%, even more preferably, at least 96%, 97%, 98% or 99% sequence identity compared to a reference sequence using the programs.
- 20 Plant Promoter A "plant promoter" is a promoter capable of initiating transcription in plant cells and can drive or facilitate transcription of a fragment of the SDF of the instant invention or a coding sequence of the SDF of the instant invention. Such promoters need not be of plant origin. For example, promoters derived from plant viruses, such as the CaMV35S promoter or from *Agrobacterium tumefaciens* such as the T-DNA promoters, can be plant promoters. A typical example of a plant promoter of plant origin is the maize ubiquitin-1 (ubi-1) promoter known to those of skill.
- 30 Promoter: The term "promoter," as used herein, refers to a region of sequence determinants located upstream from the start of transcription of a gene and which are involved in recognition and binding of RNA polymerase and other proteins to initiate and modulate transcription. A basal promoter is the minimal sequence necessary for assembly of a transcription complex required for transcription initiation. Basal promoters frequently include a

TATA box" element usually located between 15 and 35 nucleotides upstream from the site of initiation of transcription. Basal promoters also sometimes include a "CCAAT box" element (typically a sequence CCAAT) and/or a GGGCG sequence, usually located between 40 and 200 nucleotides, preferably 60 to 120 nucleotides, upstream from the start site of transcription.

Public sequence: The term "public sequence," as used in the context of the instant application, refers to any sequence that has been deposited in a publicly accessible database. This term encompasses both amino acid and nucleotide sequences. Such sequences are publicly accessible, for example, on the BLAST databases on the NCBI FTP web site (accessible at ncbi.nlm.gov/blast). The database at the NCBI FTP site utilizes "gi" numbers assigned by NCBI as a unique identifier for each sequence in the databases, thereby providing a non-redundant database for sequence from various databases, including GenBank, EMBL, DDBJ, (DNA Database of Japan) and PDB (Brookhaven Protein Data Bank).

Regulatory Sequence The term "regulatory sequence," as used in the current invention, refers to any nucleotide sequence that influences transcription or translation initiation and rate, and stability and/or mobility of the transcript or polypeptide product. Regulatory sequences include, but are not limited to, promoters, promoter control elements, protein binding sequences, 5' and 3' UTRs, transcriptional start site, termination sequence, polyadenylation sequence, introns, certain sequences within a coding sequence, etc.

Related Sequences: Related sequences" refer to either a polypeptide or a nucleotide sequence that exhibits some degree of sequence similarity with a sequence described in Table

Scaffold Attachment Region (SAR) As used herein, "scaffold attachment region" is a DNA sequence that anchors chromatin to the nuclear matrix or scaffold to generate loop domains that can have either a transcriptionally active or inactive structure (Spiker and Thompson (1996) Plant Physiol. 110: 15-21).

Sequence-determined DNA fragments (SDFs) "Sequence-determined DNA fragments" as used in the current invention are isolated sequences of genes, fragments of genes,

intergenic regions or contiguous DNA from plant genomic DNA or cDNA or RNA the sequence of which has been determined.

Signal Peptide A "signal peptide" as used in the current invention is an amino acid sequence that targets the protein for secretion, for transport to an intracellular compartment or organelle or for incorporation into a membrane. Signal peptides are indicated in the tables and a more detailed description located below.

Specific Promoter In the context of the current invention, "specific promoters" refers to a subset of inducible promoters that have a high preference for being induced in a specific tissue or cell and/or at a specific time during development of an organism. By "high preference" is meant at least 3-fold, preferably 5-fold, more preferably at least 10-fold still more preferably at least 20-fold, 50-fold or 100-fold increase in transcription in the desired tissue over the transcription in any other tissue. Typical examples of temporal and/or tissue specific promoters of plant origin that can be used with the polynucleotides of the present invention, are: PTA29, a promoter which is capable of driving gene transcription specifically in tapetum and only during anther development (Koltonow et al., *Plant Cell* 2:1201 (1990); RCc2 and RCc3, promoters that direct root-specific gene transcription in rice (Xu et al., *Plant Mol. Biol.* 27:237 (1995); TobRB27, a root-specific promoter from tobacco (Yamamoto et al., *Plant Cell* 3:371 (1991)). Examples of tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues or organs, such as root, ovule, fruit, seeds, or flowers. Other suitable promoters include those from genes encoding storage proteins or the lipid body membrane protein, oleosin. A few root-specific promoters are noted above.

Stringency "Stringency" as used herein is a function of probe length, probe composition (G + C content), and salt concentration, organic solvent concentration, and temperature of hybridization or wash conditions. Stringency is typically compared by the parameter T_m , which is the temperature at which 50% of the complementary molecules in the hybridization are hybridized, in terms of a temperature differential from T_m . High stringency conditions are those providing a condition of $T_m - 5^\circ\text{C}$ to $T_m - 10^\circ\text{C}$. Medium or moderate stringency conditions are those providing $T_m - 20^\circ\text{C}$ to $T_m - 29^\circ\text{C}$. Low stringency conditions are those providing a condition of $T_m - 40^\circ\text{C}$ to $T_m - 48^\circ\text{C}$. The relationship of hybridization conditions to T_m (in $^\circ\text{C}$) is expressed in the mathematical equation

$$T_m = 81.5 - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G+C) - (600/N) \quad (1)$$

where N is the length of the probe. This equation works well for probes 14 to 70 nucleotides in length that are identical to the target sequence. The equation below for T_m of DNA-DNA hybrids is useful for probes in the range of 50 to greater than 500 nucleotides, and for conditions that include an organic solvent (formamide).

$$T_m = 81.5 + 16.6 \log \{ [\text{Na}^+] / (1 + 0.7[\text{Na}^+]) \} + 0.41(\%G+C) - 500/L - 0.63(\%\text{formamide}) \quad (2)$$

where L is the length of the probe in the hybrid. (P. Tijessen, "Hybridization with Nucleic Acid Probes" in Laboratory Techniques in Biochemistry and Molecular Biology, P.C. van der Vliet, ed., c. 1993 by Elsevier, Amsterdam.) The T_m of equation (2) is affected by the nature of the hybrid; for DNA-RNA hybrids T_m is 10-15°C higher than calculated, for RNA-RNA hybrids T_m is 20-25°C higher. Because the T_m decreases about 1 °C for each 1% decrease in homology when a long probe is used (Bonner et al., *J. Mol. Biol.* 81:123 (1973)), stringency conditions can be adjusted to favor detection of identical genes or related family members.

Equation (2) is derived assuming equilibrium and therefore, hybridizations according to the present invention are most preferably performed under conditions of probe excess and for sufficient time to achieve equilibrium. The time required to reach equilibrium can be shortened by inclusion of a hybridization accelerator such as dextran sulfate or another high volume polymer in the hybridization buffer.

Stringency can be controlled during the hybridization reaction or after hybridization has occurred by altering the salt and temperature conditions of the wash solutions used. The formulas shown above are equally valid when used to compute the stringency of a wash solution. Preferred wash solution stringencies lie within the ranges stated above; high stringency is 5-8°C below T_m , medium or moderate stringency is 26-29°C below T_m and low stringency is 45-48°C below T_m .

Substantially free of A composition containing A is substantially free of B when at least 85% by weight of the total A+B in the composition is A. Preferably, A comprises at least about 90% by weight of the total of A+B in the composition, more preferably at least about 95% or even 99% by weight. For example, a plant gene or DNA sequence can be considered substantially free of other plant genes or DNA sequences.

Translational start site In the context of the current invention, a "translational start site" is usually an ATG in the cDNA transcript, more usually the first ATG. A single cDNA, however, may have multiple translational start sites.

5 Transcription start site "Transcription start site" is used in the current invention to describe the point at which transcription is initiated. This point is typically located about 25 nucleotides downstream from a TFIID binding site, such as a TATA box. Transcription can initiate at one or more sites within the gene, and a single gene may have multiple transcriptional
10 start sites, some of which may be specific for transcription in a particular cell-type or tissue.

Untranslated region (UTR) A "UTR" is any contiguous series of nucleotide bases that is transcribed, but is not translated. These untranslated regions may be associated with particular functions such as increasing mRNA message stability. Examples of UTRs include,
15 but are not limited to polyadenylation signals, terminations sequences, sequences located between the transcriptional start site and the first exon (5' UTR) and sequences located between the last exon and the end of the mRNA (3' UTR).

Variant: The term "variant" is used herein to denote a polypeptide or protein or
20 polynucleotide molecule that differs from others of its kind in some way. For example, polypeptide and protein variants can consist of changes in amino acid sequence and/or charge and/or post-translational modifications (such as glycosylation, etc).

25 DETAILED DESCRIPTION OF THE INVENTION

I. Polynucleotides

Exemplified SDFs of the invention represent fragments of the genome of corn, wheat, rice, soybean or *Arabidopsis* and/or represent mRNA expressed from that genome. The isolated nucleic acid of the invention also encompasses corresponding fragments of the genome and/or
30 cDNA complement of other organisms as described in detail below.

Polynucleotides of the invention can be isolated from polynucleotide libraries using primers comprising sequence similar to those described by Table 1. See, for example, the methods described in Sambrook et al., supra.

Alternatively, the polynucleotides of the invention can be produced by chemical
5 synthesis. Such synthesis methods are described below.

It is contemplated that the nucleotide sequences presented herein may contain some small percentage of errors. These errors may arise in the normal course of determination of nucleotide sequences. Sequence errors can be corrected by obtaining seeds deposited under the accession numbers cited herein, propagating them, isolating genomic DNA or appropriate
10 mRNA from the resulting plants or seeds thereof, amplifying the relevant fragment of the genomic DNA or mRNA using primers having a sequence that flanks the erroneous sequence, and sequencing the amplification product.

I.A. Probes, Primers and Substrates

SDFs of the invention can be applied to substrates for use in array applications such
15 as, but not limited to, assays of global gene expression, for example under varying conditions of development, growth conditions. The arrays can also be used in diagnostic or forensic methods (WO95/35505, US 5,445,943 and US 5,410,270).

Probes and primers of the instant invention will hybridize to a polynucleotide comprising a sequence in Table 1. Though many different nucleotide sequences can encode
20 an amino acid sequence, the sequences of Table 1 are generally preferred for encoding polypeptides of the invention. However, the sequence of the probes and/or primers of the instant invention need not be identical to those in Table 1 or the complements thereof. For example, some variation in probe or primer sequence and/or length can allow additional family members to be detected, as well as orthologous genes and more taxonomically distant
25 related sequences. Similarly, probes and/or primers of the invention can include additional nucleotides that serve as a label for detecting the formed duplex or for subsequent cloning purposes.

Probe length will vary depending on the application. For use as primers, probes are
30 12-40 nucleotides, preferably 18-30 nucleotides long. For use in mapping, probes are preferably 50 to 500 nucleotides, preferably 100-250 nucleotides long. For Southern hybridizations, probes as long as several kilobases can be used as explained below.

The probes and/or primers can be produced by synthetic procedures such as the triester method of Matteucci et al. *J. Am. Chem. Soc.* 103:3185(1981); or according to Urdea

et al. *Proc. Natl. Acad.* 80:7461 (1981) or using commercially available automated oligonucleotide synthesizers.

5 I.B. Methods of Detection and Isolation

The polynucleotides of the invention can be utilized in a number of methods known to those skilled in the art as probes and/or primers to isolate and detect polynucleotides, including, without limitation: Southern, Northern, Branched DNA hybridization assays, polymerase chain reaction, and microarray assays, and variations thereof. Specific methods
10 given by way of examples, and discussed below include:

Hybridization

Methods of Mapping

Southern Blotting

Isolating cDNA from Related Organisms

15 Isolating and/or Identifying Orthologous Genes.

Also, the nucleic acid molecules of the invention can be used in other methods, such as high density oligonucleotide hybridizing assays, described, for example, in U.S. Pat. Nos.

6,004,753; 5,945,306; 5,945,287; 5,945,308; 5,919,686; 5,919,661; 5,919,627; 5,874,248;

5,871,973; 5,871,971; and 5,871,930; and PCT Pub. Nos. WO 9946380; WO 9933981; WO

20 9933870; WO 9931252; WO 9915658; WO 9906572; WO 9858052; WO 9958672; and WO 9810858.

B.I. Hybridization

The isolated SDFs of Table 1 of the present invention can be used as probes and/or
25 primers for detection and/or isolation of related polynucleotide sequences through hybridization. Hybridization of one nucleic acid to another constitutes a physical property that defines the subject SDF of the invention and the identified related sequences. Also, such hybridization imposes structural limitations on the pair. A good general discussion of the factors for determining hybridization conditions is provided by Sambrook et al. ("Molecular
30 Cloning, a Laboratory Manual, 2nd ed., c. 1989 by Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; *see esp.*, chapters 11 and 12). Additional considerations and details of the physical chemistry of hybridization are provided by G.H. Keller and M.M. Manak "DNA Probes", 2nd Ed. pp. 1-25, c. 1993 by Stockton Press, New York, NY.

Depending on the stringency of the conditions under which these probes and/or primers are used, polynucleotides exhibiting a wide range of similarity to those in Table 1 can be detected or isolated. When the practitioner wishes to examine the result of membrane hybridizations under a variety of stringencies, an efficient way to do so is to perform the
5 hybridization under a low stringency condition, then to wash the hybridization membrane under increasingly stringent conditions.

When using SDFs to identify orthologous genes in other species, the practitioner will preferably adjust the amount of target DNA of each species so that, as nearly as is practical,
10 the same number of genome equivalents are present for each species examined. This prevents faint signals from species having large genomes, and thus small numbers of genome equivalents per mass of DNA, from erroneously being interpreted as absence of the corresponding gene in the genome.

The probes and/or primers of the instant invention can also be used to detect or isolate
15 nucleotides that are "identical" to the probes or primers. Two nucleic acid sequences or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below.

Isolated polynucleotides within the scope of the invention also include allelic variants of
20 the specific sequences presented in Table 1. The probes and/or primers of the invention can also be used to detect and/or isolate polynucleotides exhibiting at least 80% sequence identity with the sequences of Table 1 or fragments thereof.

With respect to nucleotide sequences, degeneracy of the genetic code provides the
25 possibility to substitute at least one base of the base sequence of a gene with a different base without causing the amino acid sequence of the polypeptide produced from the gene to be changed. Hence, the DNA of the present invention may also have any base sequence that has been changed from a sequence in Table 1 by substitution in accordance with degeneracy of genetic code. References describing codon usage include: Carels *et al.*, *J. Mol. Evol.* 46: 45
30 (1998) and Fennoy *et al.*, *Nucl. Acids Res.* 21(23): 5294 (1993).

B.2. Mapping

The isolated SDF DNA of the invention can be used to create various types of genetic and physical maps of the genome of corn, Arabidopsis, soybean, rice, wheat, or other plants.

Some SDFs may be absolutely associated with particular phenotypic traits, allowing construction of gross genetic maps. While not all SDFs will immediately be associated with a phenotype, all SDFs can be used as probes for identifying polymorphisms associated with phenotypes of interest. Briefly, one method of mapping involves total DNA isolation from individuals. It is subsequently cleaved with one or more restriction enzymes, separated according to mass, transferred to a solid support, hybridized with SDF DNA and the pattern of fragments compared. Polymorphisms associated with a particular SDF are visualized as differences in the size of fragments produced between individual DNA samples after digestion with a particular restriction enzyme and hybridization with the SDF. After identification of polymorphic SDF sequences, linkage studies can be conducted. By using the individuals showing polymorphisms as parents in crossing programs, F2 progeny recombinants or recombinant inbreds, for example, are then analyzed. The order of DNA polymorphisms along the chromosomes can be determined based on the frequency with which they are inherited together versus independently. The closer two polymorphisms are together in a chromosome the higher the probability that they are inherited together. Integration of the relative positions of all the polymorphisms and associated marker SDFs can produce a genetic map of the species, where the distances between markers reflect the recombination frequencies in that chromosome segment.

The use of recombinant inbred lines for such genetic mapping is described for *Arabidopsis* by Alonso-Blanco et al. (*Methods in Molecular Biology*, vol.82, *Arabidopsis Protocols*", pp. 137-146, J.M. Martinez-Zapater and J. Salinas, eds., c. 1998 by Humana Press, Totowa, NJ) and for corn by Burr ("Mapping Genes with Recombinant Inbreds", pp. 249-254. In Freeling, M. and V. Walbot (Ed.), *The Maize Handbook*, c. 1994 by Springer-Verlag New York, Inc.: New York, NY, USA; Berlin Germany; Burr et al. *Genetics* (1998) 118: 519; Gardiner, J. et al., (1993) *Genetics* 134: 917). This procedure, however, is not limited to plants and can be used for other organisms (such as yeast) or for individual cells.

The SDFs of the present invention can also be used for simple sequence repeat (SSR) mapping. Rice SSR mapping is described by Morgante et al. (*The Plant Journal* (1993) 3: 165), Panaud et al. (*Genome* (1995) 38: 1170); Senior et al. (*Crop Science* (1996) 36: 1676), Taramino et al. (*Genome* (1996) 39: 277) and Ahn et al. (*Molecular and General Genetics* (1993) 241: 483-90). SSR mapping can be achieved using various methods. In one instance, polymorphisms are identified when sequence specific probes contained within an SDF flanking an SSR are made and used in polymerase chain reaction (PCR) assays with template DNA from two or more individuals of interest. Here, a change in the number of tandem

repeats between the SSR-flanking sequences produces differently sized fragments (U.S. Patent 5,766,847). Alternatively, polymorphisms can be identified by using the PCR fragment produced from the SSR-flanking sequence specific primer reaction as a probe against Southern blots representing different individuals (U.H. Refseth et al., (1997)

5 *Electrophoresis* 18: 1519).

Genetic and physical maps of crop species have many uses. For example, these maps can be used to devise positional cloning strategies for isolating novel genes from the mapped crop species. In addition, because the genomes of closely related species are largely syntenic (that is, they display the same ordering of genes within the genome), these maps can be used
10 to isolate novel alleles from relatives of crop species by positional cloning strategies.

The various types of maps discussed above can be used with the SDFs of the invention to identify Quantitative Trait Loci (QTLs). Many important crop traits, such as the solids content of tomatoes, are quantitative traits and result from the combined interactions of several genes. These genes reside at different loci in the genome, oftentimes on different
15 chromosomes, and generally exhibit multiple alleles at each locus. The SDFs of the invention can be used to identify QTLs and isolate specific alleles as described by de Vicente and Tanksley (*Genetics* 134:585 (1993)). In addition to isolating QTL alleles in present crop species, the SDFs of the invention can also be used to isolate alleles from the corresponding QTL of wild relatives. Transgenic plants having various combinations of QTL alleles can
20 then be created and the effects of the combinations measured. Once a desired allele combination has been identified, crop improvement can be accomplished either through biotechnological means or by directed conventional breeding programs (for review see Tanksley and McCouch, *Science* 277:1063 (1997)).

In another embodiment, the SDFs can be used to help create physical maps of the
25 genome of corn, *Arabidopsis* and related species. Where SDFs have been ordered on a genetic map, as described above, they can be used as probes to discover which clones in large libraries of plant DNA fragments in YACs, BACs, etc. contain the same SDF or similar sequences, thereby facilitating the assignment of the large DNA fragments to chromosomal positions. Subsequently, the large BACs, YACs, etc. can be ordered unambiguously by more
30 detailed studies of their sequence composition (e.g. Marra et al. (1997) *Genomic Research* 7:1072-1084) and by using their end or other sequences to find the identical sequences in other cloned DNA fragments. The overlapping of DNA sequences in this way allows large contigs of plant sequences to be built that, when sufficiently extended, provide a complete

physical map of a chromosome. Sometimes the SDFs themselves will provide the means of joining cloned sequences into a contig.

The patent publication WO95/35505 and U.S. Patents 5,445,943 and 5,410,270 describe scanning multiple alleles of a plurality of loci using hybridization to arrays of oligonucleotides. These techniques are useful for each of the types of mapping discussed above.

Following the procedures described above and using a plurality of the SDFs of the present invention, any individual can be genotyped. These individual genotypes can be used for the identification of particular cultivars, varieties, lines, ecotypes and genetically modified plants or can serve as tools for subsequent genetic studies involving multiple phenotypic traits.

B.3 Southern Blot Hybridization

The sequences from Table 1 can be used as probes for various hybridization techniques. These techniques are useful for detecting target polynucleotides in a sample or for determining whether transgenic plants, seeds or host cells harbor a gene or sequence of interest and thus might be expected to exhibit a particular trait or phenotype.

In addition, the SDFs from the invention can be used to isolate additional members of gene families from the same or different species and/or orthologous genes from the same or different species. This is accomplished by hybridizing an SDF to, for example, a Southern blot containing the appropriate genomic DNA or cDNA. Given the resulting hybridization data, one of ordinary skill in the art could distinguish and isolate the correct DNA fragments by size, restriction sites, sequence and stated hybridization conditions from a gel or from a library.

Identification and isolation of orthologous genes from closely related species and alleles within a species is particularly desirable because of their potential for crop improvement. Many important crop traits, such as the solid content of tomatoes, result from the combined interactions of the products of several genes residing at different loci in the genome. Generally, alleles at each of these loci can make quantitative differences to the trait. By identifying and isolating numerous alleles for each locus from within or different species, transgenic plants with various combinations of alleles can be created and the effects of the combinations measured. Once a more favorable allele combination has been identified, crop improvement can be accomplished either through biotechnological means or by directed conventional breeding programs (Tanksley et al. *Science* 277:1063(1997)).

The results from hybridizations of the SDFs of the invention to, for example, Southern blots containing DNA from another species can also be used to generate restriction fragment maps for the corresponding genomic regions. These maps provide additional
5 information about the relative positions of restriction sites within fragments, further distinguishing mapped DNA from the remainder of the genome.

Physical maps can be made by digesting genomic DNA with different combinations of restriction enzymes.

Probes for Southern blotting to distinguish individual restriction fragments can range
10 in size from 15 to 20 nucleotides to several thousand nucleotides. More preferably, the probe is 100 to 1,000 nucleotides long for identifying members of a gene family when it is found that repetitive sequences would complicate the hybridization. For identifying an entire corresponding gene in another species, the probe is more preferably the length of the gene, typically 2,000 to 10,000 nucleotides, but probes 50-1,000 nucleotides long might be used.
15 Some genes, however, might require probes up to 1,500 nucleotides long or overlapping probes constituting the full-length sequence to span their lengths.

Also, while it is preferred that the probe be homogeneous with respect to its sequence, it is not necessary. For example, as described below, a probe representing members of a gene family having diverse sequences can be generated using PCR to amplify genomic DNA or
20 RNA templates using primers derived from SDFs that include sequences that define the gene family.

For identifying corresponding genes in another species, the next most preferable probe is a cDNA spanning the entire coding sequence, which allows all of the mRNA-coding fragment of the gene to be identified. Probes for Southern blotting can easily be generated
25 from SDFs by making primers having the sequence at the ends of the SDF and using corn or *Arabidopsis* genomic DNA as a template. In instances where the SDF includes sequence conserved among species, primers including the conserved sequence can be used for PCR with genomic DNA from a species of interest to obtain a probe.

Similarly, if the SDF includes a domain of interest, that fragment of the SDF can be used to
30 make primers and, with appropriate template DNA, used to make a probe to identify genes containing the domain. Alternatively, the PCR products can be resolved, for example by gel electrophoresis, and cloned and/or sequenced. Using Southern hybridization, the variants of the domain among members of a gene family, both within and across species, can be examined.

B.4.1 Isolating DNA from Related Organisms

The SDFs of the invention can be used to isolate the corresponding DNA from other organisms. Either cDNA or genomic DNA can be isolated. For isolating genomic DNA, a lambda, cosmid, BAC or YAC, or other large insert genomic library from the plant of interest
5 can be constructed using standard molecular biology techniques as described in detail by Sambrook et al. 1989 (Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York) and by Ausubel et al. 1992 (Current Protocols in Molecular Biology, Greene Publishing, New York).

To screen a phage library, for example, recombinant lambda clones are plated out on
10 appropriate bacterial medium using an appropriate *E. coli* host strain. The resulting plaques are lifted from the plates using nylon or nitrocellulose filters. The plaque lifts are processed through denaturation, neutralization, and washing treatments following the standard protocols outlined by Ausubel et al. (1992). The plaque lifts are hybridized to either radioactively
15 labeled or non-radioactively labeled SDF DNA at room temperature for about 16 hours, usually in the presence of 50% formamide and 5X SSC (sodium chloride and sodium citrate) buffer and blocking reagents. The plaque lifts are then washed at 42°C with 1% Sodium Dodecyl Sulfate (SDS) and at a particular concentration of SSC. The SSC concentration used is dependent upon the stringency at which hybridization occurred in the initial Southern blot
20 analysis performed. For example, if a fragment hybridized under medium stringency (e.g., $T_m - 20^\circ\text{C}$), then this condition is maintained or preferably adjusted to a less stringent condition (e.g., $T_m - 30^\circ\text{C}$) to wash the plaque lifts. Positive clones show detectable hybridization e.g., by exposure to X-ray films or chromogen formation. The positive clones are then subsequently isolated for purification using the same general protocol outlined
25 above. Once the clone is purified, restriction analysis can be conducted to narrow the region corresponding to the gene of interest. The restriction analysis and succeeding subcloning steps can be done using procedures described by, for example Sambrook et al. (1989) cited above.

The procedures outlined for the lambda library are essentially similar to those used for YAC library screening, except that the YAC clones are harbored in bacterial colonies. The
30 YAC clones are plated out at reasonable density on nitrocellulose or nylon filters supported by appropriate bacterial medium in petri plates. Following the growth of the bacterial clones, the filters are processed through the denaturation, neutralization, and washing steps following

the procedures of Ausubel et al. 1992. The same hybridization procedures for lambda library screening are followed.

To isolate cDNA, similar procedures using appropriately modified vectors are employed. For instance, the library can be constructed in a lambda vector appropriate for cloning cDNA such as λ gt11. Alternatively, the cDNA library can be made in a plasmid vector. cDNA for cloning can be prepared by any of the methods known in the art, but is preferably prepared as described above. Preferably, a cDNA library will include a high proportion of full-length clones.

B. 5. Isolating and/or Identifying Orthologous Genes

Probes and primers of the invention can be used to identify and/or isolate

- 5 polynucleotides related to those in Table 1. Related polynucleotides are those that are native to other plant organisms and exhibit either similar sequence or encode polypeptides with similar biological activity. One specific example is an orthologous gene. Orthologous genes have the same functional activity. As such, orthologous genes may be distinguished from homologous genes. The percentage of identity is a function of evolutionary separation and, in closely related
10 species, the percentage of identity can be 98 to 100%. The amino acid sequence of a protein encoded by an orthologous gene can be less than 75% identical, but tends to be at least 75% or at least 80% identical, more preferably at least 90%, most preferably at least 95% identical to the amino acid sequence of the reference protein.

- To find orthologous genes, the probes are hybridized to nucleic acids from a species of interest
15 under low stringency conditions, preferably one where sequences containing as much as 40-45% mismatches will be able to hybridize. This condition is established by $T_m - 40^\circ\text{C}$ to $T_m - 48^\circ\text{C}$ (see below). Blots are then washed under conditions of increasing stringency. It is preferable that the wash stringency be such that sequences that are 85 to 100% identical will hybridize. More preferably, sequences 90 to 100% identical will hybridize and most preferably only
20 sequences greater than 95% identical will hybridize. One of ordinary skill in the art will recognize that, due to degeneracy in the genetic code, amino acid sequences that are identical can be encoded by DNA sequences as little as 67% identical or less. Thus, it is preferable, for example, to make an overlapping series of shorter probes, on the order of 24 to 45 nucleotides, and individually hybridize them to the same arrayed library to avoid the problem of degeneracy
25 introducing large numbers of mismatches.

As evolutionary divergence increases, genome sequences also tend to diverge. Thus, one of skill will recognize that searches for orthologous genes between more divergent species will require the use of lower stringency conditions compared to searches between closely related species. Also, degeneracy of the genetic code is more of a problem for searches in the genome of a species more distant evolutionarily from the species that is the source of the SDF probe sequences.

Therefore the method described in Bouckaert et al., U.S. Ser. No. 60/121,700 Atty. Dkt. No. 2750-117P, Client Dkt. No. 00010.001, filed February 25, 1999, hereby incorporated in its entirety by reference, can be applied to the SDFs of the present invention to isolate related genes from plant species which do not hybridize to the corn *Arabidopsis*, soybean, rice, wheat, and other plant sequences of Table 1.

Identification of the relationship of nucleotide or amino acid sequences among plant species can be done by comparing the nucleotide or amino acid sequences of SDFs of the present application with nucleotide or amino acid sequences of other SDFs such as those present in applications listed in the table below:

Country	Attorney No	Client No	Filed	Application No
United States	2750-0301P	80002.001	9/4/98	60/099,672
United States	2750-0300P	80001.001	9/4/98	60/099,671
United States	2750-0302P	80003.001	9/11/98	60/099,933
United States	2750-0304P	80004.001	9/17/98	60/100,864
United States	2750-0305P	80005.001	9/18/98	60/101,042
United States	2750-0306P	80006.001	9/21/98	60/101,255
United States	2750-0307P	80007.001	9/24/98	60/101,682
United States	2750-0308P	80008.001	9/30/98	60/102,533
United States	2750-0309P	80009.001	9/30/98	60/102,460
United States	2750-0311P	80011.001	10/5/98	60/103,141
United States	2750-0310P	80010.001	10/5/98	60/103,116
United States	2750-0312P	80012.001	10/6/98	60/103,215
United States	2750-0313P	80013.001	10/8/98	60/103,554
United States	2750-0314P	80014.001	10/9/98	60/103,574
United States	2750-0315P	80015.001	10/13/98	60/103,907
United States	2750-0316P	80016.001	10/14/98	60/104,268
United States	2750-0317P	80017.001	10/16/98	60/104,680
United States	2750-0318P	80018.001	10/19/98	60/104,828
United States	2750-0319P	80019.001	10/20/98	60/105,008
United States	2750-0320P	80020.001	10/21/98	60/105,142
United States	2750-0321P	80021.001	10/22/98	60/105,533
United States	2750-0322P	80022.001	10/26/98	60/105,571
United States	2750-0323P	80023.001	10/27/98	60/105,815
United States	2750-0324P	80024.001	10/29/98	60/106,105
United States	2750-0325P	80025.001	10/30/98	60/106,218
United States	2750-0326P	80026.001	11/2/98	60/106,685
United States	2750-0327P	80027.001	11/6/98	60/107,282
United States	2750-0329P	80029.001	11/9/98	60/107,719

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United States	2750-0328P	80028.001	11/9/98	60/107.720
United States	2750-0330P	80030.001	11/10/98	60/107.836
United States	2750-0331P	80031.001	11/12/98	60/108.190
United States	2750-0332P	80032.001	11/16/98	60/108.526
United States	2750-0333P	80033.001	11/17/98	60/108.901
United States	2750-0335P	80035.001	11/19/98	60/109.127
United States	2750-0334P	80034.001	11/19/98	60/109.124
United States	2750-0336P	80036.001	11/20/98	60/109.267
United States	2750-0337P	80037.001	11/23/98	60/109.594
United States	2750-0339P	80039.001	11/25/98	60/110.050
United States	2750-0338P	80038.001	11/25/98	60/110.053
United States	2750-0340P	80040.001	11/27/98	60/110.158
United States	2750-0341P	80041.001	11/30/98	60/110.263
United States	2750-0342P	80042.001	12/1/98	60/110.495
United States	2750-0343P	80043.001	12/2/98	60/110.626
United States	2750-0344P	80044.001	12/3/98	60/110.701
United States	2750-0345P	80045.001	12/7/98	60/111.339
United States	2750-0346P	80046.001	12/9/98	60/111.589
United States	2750-0347P	80047.001	12/10/98	60/111.782
United States	2750-0348P	80048.001	12/11/98	60/111.812
United States	2750-0349P	80049.001	12/14/98	60/112.096
United States	2750-0350P	80050.001	12/15/98	60/112.224
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United States	2750-0358P	80058.001	12/29/98	60/114.384
United States	2750-0359P	80059.001	12/30/98	60/114.455
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United States	2750-0366P	80066.001	1/7/99	60/115.156
United States	2750-0365P	80065.001	1/7/99	60/115.155
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United States	2750-0373P	80073.001	1/13/99	60/115.847
United States	2750-0374P	80074.001	1/14/99	60/115.905
United States	2750-0375P	80075.001	1/15/99	60/116.383
United States	2750-0376P	80076.001	1/15/99	60/116.384
United States	2750-0378P	80078.001	1/19/99	60/116.340
United States	2750-0377P	80077.001	1/19/99	60/116.329
United States	2750-0380P	80080.001	1/21/99	60/116.672

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United States	2750-0379P	80079.001	1/21/99	60/116.674
United States	2750-0382P	80082.001	1/22/99	60/116.962
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United States	2750-0383P	80083.001	1/28/99	60/117.756
United States	2750-0384P	80084.001	2/3/99	60/118.672
United States	2750-0385P	80085.001	2/4/99	60/118.808
United States	2750-0386P	80086.001	2/5/99	60/118.778
United States	2750-0387P	80087.001	2/8/99	60/119.029
United States	2750-0388P	80088.001	2/9/99	60/119.332
United States	2750-0389P	80089.001	2/10/99	60/119.462
United States	2750-0391P	80091.001	2/12/99	60/119.922
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United States	2750-0395P	80095.001	2/22/99	60/121.072
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United States	2750-0397P	80097.001	2/24/99	60/121.470
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United States	2750-0390P	80090.001	2/25/99	60/121.825
United States	2750-0399P	80099.001	2/26/99	60/122.107
United States	2750-0400P	80100.001	3/1/99	60/122.266
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United States	2750-0402P	80102.001	3/3/99	60/122.611
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United States	2750-0426P	80126.001	5/4/99	60/132.484
United States	2750-0427P	80127.001	5/5/99	60/132.485
United States	2750-0428P	91002.001	5/6/99	60/132.487
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United States	2750-0430P	80130.001	5/7/99	60/132.863

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United States	2750-0431P	80131.001	5/11/99	60/134,256
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United States	2750-0432P	91006.001	5/14/99	60/134,370
United States	2750-0434P	80116.001	5/14/99	60/134,219
United States	2750-0435P	80117.001	5/14/99	60/134,218
United States	2750-0436P	91007.001	5/18/99	60/134,768
United States	2750-0437P	91008.001	5/19/99	60/134,941
United States	2750-0438P	91009.001	5/20/99	60/135,124
United States	2750-0439P	91010.001	5/21/99	60/135,353
United States	2750-0440P	91011.001	5/24/99	60/135,629
United States	2750-0441P	91012.001	5/25/99	60/136,021
United States	2750-0442P	91013.001	5/27/99	60/136,392
United States	2750-0444P	91014.001	5/28/99	60/136,782
United States	2750-0445P	91015.001	6/1/99	60/137,222
United States	2750-0446P	91016.001	6/3/99	60/137,528
United States	2750-0447P	91017.001	6/4/99	60/137,502
United States	2750-0449P	91018.001	6/7/99	60/137,724
United States	2750-0450P	91019.001	6/8/99	60/138,094
United States	2750-0458P	00033.002	6/10/99	60/138,847
United States	2750-0457P	00033.001	6/10/99	60/138,540
United States	2750-0463P	00034.001	6/14/99	60/139,119
United States	2750-0462P	80132.012	6/16/99	60/139,452
United States	2750-0461P	80132.011	6/16/99	60/139,453
United States	2750-0464P	00037.001	6/17/99	60/139,492
United States	2750-0465P	00038.001	6/18/99	60/139,763
United States	2750-0466P	00039.001	6/18/99	60/139,750
United States	2750-0460P	80132.010	6/18/99	60/139,455
United States	2750-0459P	80132.009	6/18/99	60/139,463
United States	2750-0443P	80132.001	6/18/99	60/139,458
United States	2750-0455P	80132.007	6/18/99	60/139,460
United States	2750-0454P	80132.006	6/18/99	60/139,457
United States	2750-0453P	80132.005	6/18/99	60/139,462
United States	2750-0452P	80132.004	6/18/99	60/139,461
United States	2750-0451P	80132.003	6/18/99	60/139,459
United States	2750-0448P	80132.002	6/18/99	60/139,454
United States	2750-0456P	80132.008	6/18/99	60/139,456
United States	2750-0467P	00042.001	6/21/99	60/139,817
United States	2750-0468P	00043.001	6/22/99	60/139,899
United States	2750-0469P	00044.001	6/23/99	60/140,354
United States	2750-0470P	00042.002	6/23/99	60/140,353
United States	2750-0471P	00045.001	6/24/99	60/140,695
United States	2750-0472P	00046.001	6/28/99	60/140,823
United States	2750-0473P	00048.001	6/29/99	60/140,991
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United States	2750-0475P	00050.001	7/1/99	60/141,842
United States	2750-0476P	00051.001	7/1/99	60/142,154
United States	2750-0477P	00052.001	7/2/99	60/142,055
United States	2750-0478P	00053.001	7/6/99	60/142,390
United States	2750-0479P	00054.001	7/8/99	60/142,803
United States	2750-0480P	00058.001	7/9/99	60/142,920
United States	2750-0481P	00059.001	7/12/99	60/142,977

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United States	2750-0482P	00060.001	7/13/99	60/143,542
United States	2750-0489P	00061.001	7/14/99	60/143,624
United States	2750-0490P	00062.001	7/15/99	60/144,005
United States	2750-0485P	80134.003	7/16/99	60/144,086
United States	2750-0486P	80134.004	7/16/99	60/144,085
United States	2750-0495P	80134.013	7/19/99	60/144,335
United States	2750-0494P	80134.010	7/19/99	60/144,333
United States	2750-0497P	00064.001	7/19/99	60/144,325
United States	2750-0492P	80134.008	7/19/99	60/144,331
United States	2750-0496P	80134.014	7/19/99	60/144,334
United States	2750-0488P	80134.006	7/19/99	60/144,332
United States	2750-0500P	00065.001	7/20/99	60/144,632
United States	2750-0502P	80135.002	7/20/99	60/144,884
United States	2750-0499P	80134.012	7/20/99	60/144,352
United States	2750-0484P	80134.002	7/21/99	60/145,086
United States	2750-0483P	80134.001	7/21/99	60/145,088
United States	2750-0503P	00066.001	7/21/99	60/144,814
United States	2750-0487P	80134.005	7/22/99	60/145,089
United States	2750-0491P	80134.007	7/22/99	60/145,085
United States	2750-0493P	80134.009	7/22/99	60/145,087
United States	2750-0504P	00067.001	7/22/99	60/145,192
United States	2750-0498P	80134.011	7/23/99	60/145,145
United States	2750-0505P	00069.001	7/23/99	60/145,218
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United States	2750-0506P	00070.001	7/26/99	60/145,276
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United States	2750-0508P	80136.002	7/27/99	60/145,919
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United States	2750-0511P	80137.001	8/2/99	60/146,388
United States	2750-0512P	80137.002	8/2/99	60/146,389
United States	2750-0513P	00073.001	8/2/99	60/146,386
United States	2750-0514P	00074.001	8/3/99	60/147,038
United States	2750-0517P	80138.002	8/4/99	60/147,302
United States	2750-0515P	00076.001	8/4/99	60/147,204
United States	2750-0518P	00077.001	8/5/99	60/147,260
United States	2750-0519P	80136.003	8/5/99	60/147,192
United States	2750-0516P	80138.001	8/6/99	60/147,303
United States	2750-0520P	00079.001	8/6/99	60/147,416
United States	2750-0521P	00080.001	8/9/99	60/147,493
United States	2750-0523P	80139.002	8/9/99	60/147,935
United States	2750-0522P	80139.001	8/10/99	60/148,171
United States	2750-0524P	00081.001	8/11/99	60/148,319
United States	2750-0528P	80141.004	8/12/99	60/148,337
United States	2750-0530P	00082.001	8/12/99	60/148,341
United States	2750-0527P	80141.003	8/12/99	60/148,340
United States	2750-0525P	80141.001	8/12/99	60/148,347
United States	2750-0526P	80141.002	8/12/99	60/148,342
United States	2750-0532P	80142.002	8/13/99	60/148,684
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United States	2750-0531P	80142.001	8/16/99	60/149,368

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United States	2750-0534P	80001.003	8/17/99	60/149,928
United States	2750-0536P	80001.005	8/17/99	60/149,925
United States	2750-0533P	80001.002	8/17/99	60/149,927
United States	2750-0537P	00084.001	8/17/99	60/149,175
United States	2750-0535P	80001.004	8/17/99	60/149,926
United States	2750-0538P	00085.001	8/18/99	60/149,426
United States	2750-0539P	00086.001	8/20/99	60/149,722
United States	2750-0541P	80143.002	8/20/99	60/149,929
United States	2750-0542P	00087.001	8/20/99	60/149,723
United States	2750-0540P	80143.001	8/23/99	60/149,930
United States	2750-0543P	00088.001	8/23/99	60/149,902
United States	2750-0544P	00089.001	8/25/99	60/150,566
United States	2750-0547P	00090.001	8/26/99	60/150,884
United States	2750-0548P	00091.001	8/27/99	60/151,080
United States	2750-0546P	80144.002	8/27/99	60/151,066
United States	2750-0545P	80144.001	8/27/99	60/151,065
United States	2750-0549P	00092.001	8/30/99	60/151,303
United States	2750-0552P	00093.001	8/31/99	60/151,438
United States	2750-0553P	00094.001	9/1/99	60/151,930
United States	2750-0550P	80001.006	9/3/99	09/391,631
International	2750-0551F(PC)	80001.100	9/3/99	99/204,38
United States	2750-0554P	00095.001	9/7/99	60/152,363
United States	2750-0555P	00096.001	9/10/99	60/153,070
United States	2750-0556P	00098.001	9/13/99	60/153,758
United States	2750-0557P	00099.001	9/15/99	60/154,018
United States	2750-0558P	00101.001	9/16/99	60/154,039
United States	2750-0559P	00102.001	9/20/99	60/154,779
United States	2750-0560P	00103.001	9/22/99	60/155,139
United States	2750-0561P	00104.001	9/23/99	60/155,486
United States	2750-0562P	00105.001	9/24/99	60/155,659
United States	2750-0563P	00106.001	9/28/99	60/156,458
United States	2750-0564P	00107.001	9/29/99	60/156,596
United States	2750-0570P	00108.001	10/4/99	60/157,117
United States	2750-0566P	80010.003	10/5/99	09/412,922
United States	2750-0571P	00109.001	10/5/99	60/157,753
International	2750-0569F(PC)	80010.102	10/5/99	99/228,53
International	2750-0567F(PC)	80010.100	10/5/99	99/228,55
United States	2750-0565P	80010.002	10/5/99	09/413,198
International	2750-0568F(PC)	80010.101	10/5/99	99/228,54
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United States	2750-0576P	00112.001	10/8/99	60/158,232
United States	2750-0577P	00113.001	10/12/99	60/158,369
United States	2750-0583P	80148.002	10/13/99	60/159,294
United States	2750-0574P	80145.002	10/13/99	60/159,295
United States	2750-0579P	80146.002	10/13/99	60/159,293
United States	2750-0581P	80147.002	10/14/99	60/159,637
United States	2750-0578P	80146.001	10/14/99	60/159,331
United States	2750-0582P	80148.001	10/14/99	60/159,329
United States	2750-0580P	80147.001	10/14/99	60/159,638
United States	2750-0573P	80145.001	10/14/99	60/159,330

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United States	2750-0637P	00134.001	11/15/99	60/164,927
United States	2750-0638P	80167.001	11/15/99	60/164,929
United States	2750-0639P	80167.002	11/15/99	60/164,926
United States	2750-0640P	00135.001	11/16/99	60/165,669
United States	2750-0641P	80168.001	11/16/99	60/165,671
United States	2750-0642P	80168.002	11/16/99	60/165,661
United States	2750-0645P	80169.002	11/17/99	60/165,911
United States	2750-0643P	00136.001	11/17/99	60/165,919
United States	2750-0644P	80169.001	11/17/99	60/165,918
United States	2750-0648P	80170.002	11/18/99	60/166,158
United States	2750-0646P	00137.001	11/18/99	60/166,157
United States	2750-0647P	80170.001	11/18/99	60/166,173
United States	2750-0649P	00139.001	11/19/99	60/166,419
United States	2750-0650P	80171.001	11/19/99	60/166,411
United States	2750-0651P	80171.002	11/19/99	60/166,412
United States	2750-0652P	00140.001	11/22/99	60/166,733
United States	2750-0653P	80172.001	11/22/99	60/166,750
United States	2750-0655P	80173.002	11/23/99	60/167,362
United States	2750-0658P	80174.002	11/24/99	60/167,235
United States	2750-0654P	80173.001	11/24/99	60/167,382
United States	2750-0656P	00141.001	11/24/99	60/167,233
United States	2750-0657P	80174.001	11/24/99	60/167,234
United States	2750-0659P	00142.001	11/30/99	60/167,904
United States	2750-0661P	80175.002	11/30/99	60/167,902
United States	2750-0660P	80175.001	11/30/99	60/167,908
United States	2750-0664P	80176.001	12/1/99	60/168,233
United States	2750-0662P	80042.002	12/1/99	09/451,320
United States	2750-0665P	80176.002	12/1/99	60/168,231
United States	2750-0663P	00143.001	12/1/99	60/168,232
United States	2750-0667P	80177.001	12/2/99	60/168,549
United States	2750-0666P	00144.001	12/2/99	60/168,546
United States	2750-0668P	80177.002	12/2/99	60/168,548
United States	2750-0670P	80178.001	12/3/99	60/168,673
United States	2750-0669P	00145.001	12/3/99	60/168,675
United States	2750-0671P	80178.002	12/3/99	60/168,674
United States	2750-0673P	80179.001	12/7/99	60/169,278
United States	2750-0674P	80179.002	12/7/99	60/169,302
United States	2750-0672P	00147.001	12/7/99	60/169,298
United States	2750-0676P	80180.002	12/8/99	60/169,691
United States	2750-0675P	80180.001	12/8/99	60/169,692
United States	2750-0678P	80181.001	12/16/99	60/171,114
United States	2750-0679P	80181.002	12/16/99	60/171,098
United States	2750-0677P	00149.001	12/16/99	60/171,107
United States	2750-0683P	80060.002	1/4/00	09/478,081
International	2750-0686F(PC)	80070.100	1/7/00	00/004,66
United States	2750-0684P	80070.002	1/7/00	09/479,221
United States	2750-0685P	80183.002	1/19/00	60/176,867
United States	2750-0688P	80184.002	1/19/00	60/176,910
United States	2750-0681P	80182.002	1/19/00	60/176,866

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Country	Attorney No	Client No	Filed	Application No
United States	2750-0689P	00152.001	1/26/00	60/178,166
United States	2750-0680P	80182.001	1/27/00	60/178,544
United States	2750-0682P	80183.001	1/27/00	60/178,546
United States	2750-0690P	00153.001	1/27/00	60/178,547
United States	2750-0691P	80185.001	1/27/00	60/177,666
United States	2750-0687P	80184.001	1/27/00	60/178,545
United States	2750-0692P	00155.001	1/28/00	60/178,754
United States	2750-0693P	80186.001	1/28/00	60/178,755
United States	2750-0695P	00157.001	2/1/00	60/179,395
United States	2750-0696P	80187.001	2/1/00	60/179,388
United States	2750-0694P	80084.002	2/3/00	09/497,191
United States	2750-0697P	00158.001	2/3/00	60/180,039
United States	2750-0698P	80188.001	2/3/00	60/180,139
United States	2750-0700P	80189.001	2/4/00	60/180,207
United States	2750-0699P	00159.001	2/4/00	60/180,206
United States	2750-0701P	00160.001	2/7/00	60/180,695
United States	2750-0702P	80190.001	2/7/00	60/180,696
United States	2750-0703P	00161.001	2/9/00	60/181,228
United States	2750-0704P	80191.001	2/9/00	60/181,214
United States	2750-0705P	00162.001	2/10/00	60/181,476
United States	2750-0706P	80192.001	2/10/00	60/181,551
United States	2750-0707P	00163.001	2/15/00	60/182,477
United States	2750-0708P	80193.001	2/15/00	60/182,516
United States	2750-0712P	00164.001	2/15/00	60/182,512
United States	2750-0713P	80194.001	2/15/00	60/182,478
United States	2750-0714P	00165.001	2/17/00	60/183,166
United States	2750-0715P	80195.001	2/17/00	60/183,165
United States	2750-0716P	00167.001	2/24/00	60/184,667
United States	2750-0717P	80196.001	2/24/00	60/184,658
Mexico	2750-0709F(MX)	80090.101	2/25/00	00/001,973
United States	2750-0719P	00168.001	2/25/00	60/185,118
United States	2750-0718P	91022.001	2/25/00	60/185,140
United States	2750-0720P	80197.001	2/25/00	60/185,119
Canada	2750-0709F(CA)	80090.102	2/25/00	23/006,92
Europe	2750-0709F(EP)	80090.103	2/25/00	00/301,439
United States	2750-0709P	80090.002	2/25/00	09/513,996
United States	2750-0721P	91023.001	2/28/00	60/185,398
United States	2750-0722P	00169.001	2/28/00	60/185,396
United States	2750-0723P	80198.001	2/28/00	60/185,397
United States	2750-0724P	91024.001	2/29/00	60/185,750
United States	2750-0726P	80199.001	3/1/00	60/186,296
United States	2750-0727P	91025.001	3/1/00	60/186,277
United States	2750-0725P	00170.001	3/1/00	
United States	2750-0710P	80100.002	3/1/00	09/517,537
United States	2750-0729P	00172.001	3/2/00	60/186,386
United States	2750-0730P	80201.001	3/2/00	60/186,387
United States	2750-0711P	00171.001	3/2/00	60/186,390
United States	2750-0728P	80200.001	3/2/00	60/187,178
United States	2750-0731P	91026.001	3/3/00	60/186,670
United States	2750-0732P	00173.001	3/3/00	60/186,748
United States	2750-0733P	80202.001	3/3/00	60/186,669

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Country	Attorney No	Client No	Filed	Application No
United States	2750-0734P	00174.001	3/7/00	60/187,378
United States	2750-0735P	91027.001	3/7/00	60/187,379
United States	2750-0736P	00175.001	3/8/00	60/187,896
United States	2750-0737P	80203.001	3/8/00	60/187,888
United States	2750-0738P	91028.001	3/9/00	60/187,985
United States	2750-0741P	91030.001	3/10/00	
United States	2750-0742P	00178.001	3/10/00	60/188,185
United States	2750-0743P	80205.001	3/10/00	60/188,175
United States	2750-0739P	00177.001	3/10/00	60/188,187
United States	2750-0740P	80204.001	3/10/00	60/188,186
United States	2750-0744P	91031.001	3/13/00	60/188,687
United States	2750-0745P	00179.001	3/14/00	60/189,080
United States	2750-0746P	80206.001	3/14/00	60/189,052
United States	2750-0748P	00180.001	3/15/00	60/189,461
United States	2750-0749P	80207.001	3/15/00	60/189,462
United States	2750-0747P	91032.001	3/15/00	60/189,460
United States	2750-0753P	80211.001	3/16/00	60/190,121
United States	2750-0757P	91034.001	3/16/00	60/189,965
United States	2750-0754P	91033.001	3/16/00	60/189,958
United States	2750-0752P	80210.001	3/16/00	60/189,948
United States	2750-0755P	00181.001	3/16/00	60/189,953
United States	2750-0756P	80212.001	3/16/00	60/189,959
United States	2750-0750P	80208.001	3/16/00	60/190,120
United States	2750-0751P	80209.001	3/16/00	60/189,947
United States	2750-0762P	80214.001	3/20/00	60/190,089
United States	2750-0761P	00183.001	3/20/00	60/190,545
United States	2750-0760P	91035.001	3/20/00	60/190,060
United States	2750-0758P	00182.001	3/20/00	60/190,069
United States	2750-0759P	80213.001	3/20/00	60/190,070
United States	2750-0764P	80215.001	3/22/00	60/191,097
United States	2750-0763P	00184.001	3/22/00	60/191,084
United States	2750-0767P	80216.001	3/23/00	60/191,545
United States	2750-0766P	00185.001	3/23/00	60/191,543
United States	2750-0765P	91036.001	3/23/00	60/191,549
United States	2750-0770P	80217.001	3/24/00	60/191,825
United States	2750-0768P	91037.001	3/24/00	60/191,826
United States	2750-0769P	00186.001	3/24/00	60/191,823
United States	2750-0771P	91038.001	3/27/00	60/192,420
United States	2750-0772P	00187.001	3/27/00	60/192,421
United States	2750-0773P	80218.001	3/27/00	60/192,308
United States	2750-0776P	80219.001	3/29/00	60/192,941
United States	2750-0774P	91039.001	3/29/00	60/192,855
United States	2750-0775P	00188.001	3/29/00	60/192,940
United States	2750-0779P	80220.001	3/30/00	60/193,245
United States	2750-0777P	91040.001	3/30/00	60/193,243
United States	2750-0778P	00189.001	3/30/00	60/193,244
United States	2750-0780P	91041.001	3/31/00	60/193,469
United States	2750-0781P	00190.001	3/31/00	60/193,453
United States	2750-0782P	80221.001	3/31/00	60/193,455
United States	2750-0785P	91042.001	4/4/00	
United States	2750-0786P	00191.001	4/4/00	

Country	Attorney No	Client No	Filed	Application No
United States	2750-0787P	80222.001	4/4/00	
United States	2750-0789P	91043.001	4/5/00	
United States	2750-0790P	00192.001	4/5/00	
United States	2750-0791P	80223.001	4/5/00	
United States	2750-0792P	91044.001	4/5/00	
Europe	2750-0783F(EP)	91000.101	4/6/00	00/302.919
United States	2750-0793P	00193.001	4/6/00	
Mexico	2750-0783F(MX)	91000.100	4/6/00	00/003.391
Canada	2750-0783F(CA)	91000.102	4/6/00	
United States	2750-0783P	91000.002	4/6/00	
United States	2750-0796P	80225.001	4/6/00	
United States	2750-0784P	91045.001	4/6/00	
United States	2750-0795P	00194.001	4/6/00	
United States	2750-0794P	80224.001	4/6/00	60/194.872
United States	2750-0799P	80226.001	4/7/00	60/195.257
United States	2750-0797P	91046.001	4/7/00	
United States	2750-0798P	00195.001	4/7/00	60/195.283
United States	2750-0802P	91047.001	4/11/00	60/196.168
United States	2750-0803P	00196.001	4/11/00	
United States	2750-0801P	80227.002	4/11/00	60/196.211
United States	2750-0804P	80228.001	4/11/00	60/196.089
United States	2750-0808P	00200.001	4/12/00	60/196.485
United States	2750-0807P	80229.001	4/12/00	
United States	2750-0806P	00197.001	4/12/00	60/196.487
United States	2750-0805P	91048.001	4/12/00	60/196.483
United States	2750-0800P	80227.001	4/12/00	60/196.212
United States	2750-0809P	80230.001	4/12/00	60/196.486
United States	2750-0811P	80231.002	4/13/00	
United States	2750-0810P	80231.001	4/14/00	
United States	2750-0814P	91049.001	4/14/00	60/197.397
United States	2750-0813P	80232.002	4/17/00	60/197.871
United States	2750-0815P	00201.001	4/17/00	60/197.687
United States	2750-0819P	80234.001	4/17/00	60/197.671
United States	2750-0816P	80233.001	4/17/00	60/197.678
United States	2750-0812P	80232.001	4/17/00	60/197.870
United States	2750-0817P	91050.001	4/17/00	60/198.268
United States	2750-0818P	00202.001	4/17/00	60/198.133
United States	2750-0820P	91051.001	4/19/00	60/198.400
United States	2750-0821P	00203.001	4/19/00	60/198.386
United States	2750-0822P	80235.001	4/19/00	60/198.373
United States	2750-0823P	91052.001	4/20/00	60/198.629
United States	2750-0824P	00204.001	4/20/00	60/198.619
United States	2750-0825P	80236.001	4/20/00	60/198.623
United States	2750-0827P	00206.001	4/21/00	60/198.767
United States	2750-0828P	80237.001	4/21/00	60/198.763
United States	2750-0826P	91053.001	4/21/00	
United States	2750-0831P	80238.001	4/24/00	
United States	2750-0830P	00207.001	4/24/00	
United States	2750-0829P	91054.001	4/24/00	
United States	2750-0832P	92001.001	4/26/00	60/200.034
United States	2750-0833P	92002.001	4/26/00	

Country	Attorney No	Client No	Filed	Application No
United States	2750-0834P	00208.001	4/26/00	
United States	2750-0835P	80239.001	4/26/00	
United States	2750-0836P	00210.001	4/27/00	
United States	2750-0837P	80240.001	4/27/00	
United States	2750-0788P	80123.002	4/28/00	
United States	2750-0844P	80242.002	4/28/00	
United States	2750-0846P	80243.002	4/28/00	
United States	2750-0848P	80244.002	5/1/00	
United States	2750-0841P	92001.002	5/1/00	
United States	2750-0842P	92002.002	5/1/00	
United States	2750-0847P	80244.001	5/1/00	
United States	2750-0845P	80243.001	5/1/00	
United States	2750-0843P	80242.001	5/1/00	
United States	2750-0839P	80241.001	5/1/00	
United States	2750-0840P	91055.001	5/1/00	
United States	2750-0838P	00211.001	5/2/00	
United States	2750-0849P	91056.001	5/2/00	
United States	2750-0850P	80245.001	5/2/00	
United States	2750-0856P	91057.001	5/4/00	
United States	2750-0857P	00212.001	5/4/00	
United States	2750-0858P	80246.001	5/4/00	
United States	2750-0852P	80126.002	5/4/00	
Mexico	2750-0851F(MX)	91002.102	5/5/00	
Europe	2750-0851F(EP)	91002.101	5/5/00	
United States	2750-0859P	91058.001	5/5/00	
United States	2750-0855P	80130.002	5/5/00	
United States	2750-0854P	80129.002	5/5/00	
United States	2750-0853P	80127.002	5/5/00	
United States	2750-0860P	00213.001	5/5/00	
United States	2750-0861P	80247.001	5/5/00	
Canada	2750-0851F(CA)	91002.100	5/5/00	
United States	2750-0851P	91002.002	5/5/00	
United States	2750-0862P	00214.001	5/9/00	
United States	2750-0866P	80249.001	5/9/00	
United States	2750-0865P	00215.001	5/9/00	
United States	2750-0863P	80248.001	5/9/00	
United States	2750-0864P	91059.001	5/9/00	
United States	2750-0877P	91060.001	5/10/00	
United States	2750-0878P	00216.001	5/10/00	
United States	2750-0879P	80252.001	5/10/00	
United States	2750-0881P	00217.001	5/11/00	
United States	2750-0880P	91061.001	5/11/00	
United States	2750-0868P	80250.002	5/11/00	
United States	2750-0882P	80253.001	5/11/00	
United States	2750-0871P	80131.002	5/11/00	09/572.408
United States	2750-0867P	80250.001	5/11/00	
United States	2750-0870P	80251.002	5/11/00	
United States	2750-0869P	80251.001	5/11/00	
Europe	2750-0875F(EP)	91006.101	5/12/00	
Mexico	2750-0875F(MX)	91006.102	5/12/00	
United States	2750-0874P	80116.002	5/12/00	

Country	Attorney No	Client No	Filed	Application No
United States	2750-0873P	00025.002	5/12/00	
United States	2750-0875P	91006.002	5/12/00	
United States	2750-0883P	91062.001	5/12/00	
United States	2750-0872P	80117.002	5/12/00	
United States	2750-0885P	80254.001	5/12/00	
United States	2750-0884P	00219.001	5/12/00	
Canada	2750-0875F(CA)	91006.100	5/12/00	
United States	2750-0886P	91063.001	5/15/00	
United States	2750-0887P	00220.001	5/15/00	
United States	2750-0888P	80255.001	5/15/00	
United States	2750-0892P	80256.001	5/16/00	
United States	2750-0891P	00221.001	5/16/00	
United States	2750-0893P	00222.001	5/17/00	
United States	2750-0890P	92002.003	5/17/00	
United States	2750-0889P	92001.003	5/17/00	
United States	2750-0894P	80257.001	5/17/00	
Canada	2750-0876F(CA)	91007.100	5/18/00	
Europe	2750-0876F(EP)	91007.101	5/18/00	
Mexico	2750-0876F(MX)	91007.102	5/18/00	
United States	2750-0876P	91007.002	5/18/00	
United States	2750-0895P	00223.001	5/18/00	
United States	2750-0896P	80258.001	5/18/00	
United States	2750-0897P	00224.001	5/19/00	
United States	2750-0898P	80259.001	5/19/00	
United States	2750-0901P	80260.001	5/22/00	
United States	2750-0900P	00225.001	5/22/00	
United States	2750-0899P	91064.001	5/22/00	
United States	2750-0902P	00226.001	5/23/00	
United States	2750-0903P	80261.001	5/23/00	
United States	2750-0904P	00227.001	5/24/00	
United States	2750-0905P	80262.001	5/24/00	
United States	2750-0906P	91065.001	5/25/00	
United States	2750-0907P	00228.001	5/26/00	
United States	2750-0911P	80264.001	5/26/00	
United States	2750-0910P	00229.001	5/26/00	
United States	2750-0908P	80263.001	5/26/00	
United States	2750-0909P	91066.001	5/26/00	
United States	2750-0914P	80265.001	5/30/00	
United States	2750-0913P	00230.001	5/30/00	
United States	2750-0912P	91067.001	5/30/00	
United States	2750-0916P	80266.002	6/1/00	
United States	2750-0920P	00231.001	6/1/00	
United States	2750-0921P	80268.001	6/1/00	
United States	2750-0918P	80267.002	6/1/00	
United States	2750-0919P	91068.001	6/1/00	
United States	2750-0915P	80266.001	6/2/00	
United States	2750-0917P	80267.001	6/2/00	
United States	2750-0922P	91069.001	6/5/00	
United States	2750-0923P	00232.001	6/5/00	
United States	2750-0924P	80269.001	6/5/00	
United States	2750-0925P	91070.001	6/5/00	

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United States	2750-0926P	00233.001	6/5/00	
United States	2750-0927P	80270.001	6/5/00	
United States	2750-0931P	80271.001	6/8/00	
United States	2750-0930P	00234.001	6/8/00	
United States	2750-0929P	91071.001	6/8/00	
Canada	2750-0928F(CA)	00033.100	6/9/00	
Mexico	2750-0928F(MX)	00033.102	6/9/00	
United States	2750-0928P	00033.003	6/9/00	09/592.459
United States	2750-0933P	80272.001	6/9/00	
Mexico	2750-1037F(MX)		6/9/00	
United States	2750-0932P	00235.001	6/9/00	
Europe	2750-0928F(EP)	00033.101	6/12/00	
United States	2750-0935P	00237.001	6/13/00	
United States	2750-0936P	80273.001	6/13/00	
United States	2750-0937P	91072.001	6/13/00	
United States	2750-0934P	00034.002	6/14/00	
United States	2750-0940P	91074.001	6/15/00	
United States	2750-0939P	80274.001	6/15/00	
United States	2750-0938P	00238.001	6/15/00	
United States	2750-0943P	00039.002	6/16/00	
United States	2750-0947P	80132.016	6/16/00	
United States	2750-0954P	80132.023	6/16/00	
United States	2750-0955P	80132.024	6/16/00	
United States	2750-0945P	80132.014	6/16/00	
United States	2750-0953P	80132.022	6/16/00	
United States	2750-0952P	80132.021	6/16/00	
United States	2750-0948P	80132.017	6/16/00	
United States	2750-0944P	80132.013	6/16/00	
United States	2750-0942P	00038.002	6/16/00	
United States	2750-0946P	80132.015	6/16/00	
United States	2750-0951P	80132.020	6/16/00	
United States	2750-0941P	00037.002	6/16/00	
United States	2750-0950P	80132.019	6/16/00	
United States	2750-0949P	80132.018	6/16/00	
United States	2750-0958P	91075.001	6/19/00	
United States	2750-0956P	00239.001	6/19/00	
United States	2750-0957P	80275.001	6/19/00	
United States	2750-0961P	91076.001	6/20/00	
United States	2750-0960P	80276.001	6/20/00	
United States	2750-0959P	00240.001	6/20/00	
United States	2750-0971P	00042.003	6/21/00	
Canada	2750-0971F(CA)	00042.100	6/21/00	
Europe	2750-0971F(EP)	00042.101	6/21/00	
Mexico	2750-0971F(MX)	00042.102	6/21/00	
United States	2750-0963P	80277.001	6/22/00	
United States	2750-0964P	91077.001	6/22/00	
United States	2750-0967P	91079.001	6/22/00	
United States	2750-0966P	80278.001	6/22/00	
United States	2750-0965P	00246.001	6/22/00	
United States	2750-0972P	00043.002	6/22/00	
United States	2750-0962P	00242.001	6/22/00	

Country	Attorney No	Client No	Filed	Application No
Mexico	2750-0972F(MX)	00043.102	6/22/00	
Europe	2750-0972F(EP)	00043.101	6/22/00	
Canada	2750-0972F(CA)	00043.100	6/22/00	
Europe	2750-0973F(EP)	00044.101	6/23/00	
Canada	2750-0973F(CA)	00044.100	6/23/00	
United States	2750-0973P	00044.002	6/23/00	
Europe	2750-0975F(EP)	00045.101	6/23/00	
Canada	2750-0975F(CA)	00045.100	6/23/00	
Mexico	2750-0975F(MX)	00045.102	6/23/00	
United States	2750-0975P	00045.002	6/23/00	
Mexico	2750-0973F(MX)	00044.102	6/23/00	
United States	2750-1036P	80280.001	6/27/00	
United States	2750-1035P	00248.001	6/27/00	
United States	2750-0970P	91080.001	6/27/00	
United States	2750-0969P	80279.001	6/27/00	
United States	2750-0968P	00247.001	6/27/00	
United States	2750-1038P	00249.001	6/28/00	
Mexico	2750-0976F(MX)	00046.102	6/28/00	
United States	2750-1039P	80281.001	6/28/00	
United States	2750-0976P	00046.002	6/28/00	
Canada	2750-0976F(CA)	00046.100	6/28/00	
Europe	2750-0976F(EP)	00046.101	6/28/00	
Canada	2750-0977F(CA)	00048.100	6/29/00	
Europe	2750-0977F(EP)	00048.101	6/29/00	
Mexico	2750-0977F(MX)	00048.102	6/29/00	
United States	2750-0977P	00048.002	6/29/00	
United States	2750-0981P	00052.002	6/30/00	
Canada	2750-0980F(CA)	00051.100	6/30/00	
Mexico	2750-0980F(MX)	00051.102	6/30/00	
Europe	2750-0980F(EP)	00051.101	6/30/00	
United States	2750-0979P	00050.002	6/30/00	
Canada	2750-0979F(CA)	00050.100	6/30/00	
Europe	2750-0979F(EP)	00050.101	6/30/00	
Mexico	2750-0979F(MX)	00050.102	6/30/00	
Mexico	2750-0981F(MX)	00052.102	6/30/00	
United States	2750-0978P	00049.002	6/30/00	
Canada	2750-0978F(CA)	00049.100	6/30/00	
Europe	2750-0978F(EP)	00049.101	6/30/00	
Mexico	2750-0978F(MX)	00049.102	6/30/00	
Canada	2750-0981F(CA)	00052.100	6/30/00	
United States	2750-0980P	00051.002	6/30/00	
United States	2750-1041P	80282.001	6/30/00	
United States	2750-1040P	00250.001	6/30/00	
Europe	2750-0981F(EP)	00052.101	6/30/00	
United States	2750-1042P	00252.001	7/5/00	
United States	2750-1043P	80283.001	7/5/00	
Canada	2750-0982F(CA)	00053.100	7/6/00	
United States	2750-0982P	00053.002	7/6/00	
Mexico	2750-0982F(MX)	00053.102	7/6/00	
Europe	2750-0982F(EP)	00053.101	7/6/00	
Mexico	2750-0983F(MX)	00054.102	7/7/00	

Country	Attorney No	Client No	Filed	Application No
United States	2750-0984P	00058.002	7/7/00	
Canada	2750-0984F(CA)	00058.100	7/7/00	
Mexico	2750-0984F(MX)	00058.102	7/7/00	
United States	2750-0983P	00054.002	7/7/00	
Canada	2750-0983F(CA)	00054.100	7/7/00	
Europe	2750-0983F(EP)	00054.101	7/7/00	
Europe	2750-0984F(EP)	00058.101	7/7/00	
United States	2750-1044P	91081.001	7/11/00	
United States	2750-1045P	00253.001	7/11/00	
United States	2750-1046P	80284.001	7/11/00	
Canada	2750-0985F(CA)	00059.100	7/12/00	
United States	2750-1050P	80286.002	7/12/00	
United States	2750-1052P	80287.002	7/12/00	
Europe	2750-0985F(EP)	00059.101	7/12/00	
United States	2750-0985P	00059.002	7/12/00	
Mexico	2750-0985F(MX)	00059.102	7/12/00	
United States	2750-1054P	80288.002	7/13/00	
United States	2750-0986P	00060.002	7/13/00	
Canada	2750-0986F(CA)	00060.100	7/13/00	
Europe	2750-0986F(EP)	00060.101	7/13/00	
Mexico	2750-0986F(MX)	00060.102	7/13/00	
Europe	2750-0988F(EP)	00062.101	7/14/00	
Canada	2750-0988F(CA)	00062.100	7/14/00	
United States	2750-0988P	00062.002	7/14/00	
Mexico	2750-0987F(MX)	00061.102	7/14/00	
Europe	2750-0987F(EP)	00061.101	7/14/00	
United States	2750-0987P	00061.002	7/14/00	
Mexico	2750-0988F(MX)	00062.102	7/14/00	
Canada	2750-0987F(CA)	00061.100	7/14/00	
United States	2750-1060P	80134.017	7/14/00	
United States	2750-1048P	80285.002	7/14/00	
United States	2750-1061P	80134.018	7/14/00	

All applications listed in the table above are expressly incorporated herein by reference in their entirety and for all purposes.

- The SDFs of the invention can also be used as probes to search for genes that are related to the SDF within a species. Such related genes are typically considered to be members of a gene family. In such a case, the sequence similarity will often be concentrated into one or a few fragments of the sequence. The fragments of similar sequence that define the gene family typically encode a fragment of a protein or RNA that has an enzymatic or structural function. The percentage of identity in the amino acid sequence of the domain that defines the gene family is preferably at least 70%, more preferably 80 to 95%, most preferably 85 to 99%. To search for members of a gene family within a species, a low stringency hybridization is usually performed, but this will depend upon the size, distribution

and degree of sequence divergence of domains that define the gene family. SDFs encompassing regulatory regions can be used to identify coordinately expressed genes by using the regulatory region sequence of the SDF as a probe.

- In the instances where the SDFs are identified as being expressed from genes that confer a particular phenotype, then the SDFs can also be used as probes to assay plants of different species for those phenotypes.

I.C. Methods to Inhibit Gene Expression

The nucleic acid molecules of the present invention can be used to inhibit gene transcription and/or translation. Example of such methods include, without limitation:

- Antisense Constructs;
- Ribozyme Constructs;
- Chimeraplast Constructs;
- Co-Suppression;
- Transcriptional Silencing; and
- Other Methods of Gene Expression.

C.1 Antisense

- In some instances it is desirable to suppress expression of an endogenous or exogenous gene. A well-known instance is the FLAVOR-SAVOR™ tomato, in which the gene encoding ACC synthase is inactivated by an antisense approach, thus delaying softening of the fruit after ripening. See for example, U.S. Patent No. 5,859,330; U.S. Patent No. 5,723,766; Oeller, et al, *Science*, 254:437-439(1991); and Hamilton et al, *Nature*, 346:284-287 (1990). Also, timing of flowering can be controlled by suppression of the *FLOWERING LOCUS C (FLC)*; high levels of this transcript are associated with late flowering, while absence of *FLC* is associated with early flowering (S.D. Michaels et al., *Plant Cell* 11:949 (1999). Also, the transition of apical meristem from production of leaves with associated shoots to flowering is regulated by *TERMINAL FLOWER1*, *APETALA1* and *LEAFY*. Thus, when it is desired to induce a transition from shoot production to flowering, it is desirable to suppress *TFL1* expression (S.J. Liljegren, *Plant Cell* 11:1007 (1999)). As another instance, arrested ovule development and female sterility result from suppression of the ethylene forming enzyme but can be reversed by application of ethylene (D. De Martinis et al., *Plant*

Cell 11:1061 (1999)). The ability to manipulate female fertility of plants is useful in increasing fruit production and creating hybrids.

In the case of polynucleotides used to inhibit expression of an endogenous gene, the introduced sequence need not be perfectly identical to a sequence of the target endogenous gene.

- 5 The introduced polynucleotide sequence will typically be at least substantially identical to the target endogenous sequence.

Some polynucleotide SDFs in Table 1 represent sequences that are expressed in corn, wheat, rice, soybean *Arabidopsis* and/or other plants. Thus the invention includes using these sequences to generate antisense constructs to inhibit translation and/or degradation of
10 transcripts of said SDFs, typically in a plant cell.

To accomplish this, a polynucleotide segment from the desired gene that can hybridize to the mRNA expressed from the desired gene (the "antisense segment") is operably linked to a promoter such that the antisense strand of RNA will be transcribed when the construct is present in a host cell. A regulated promoter can be used in the construct to control transcription of the
15 antisense segment so that transcription occurs only under desired circumstances.

The antisense segment to be introduced generally will be substantially identical to at least a fragment of the endogenous gene or genes to be repressed. The sequence, however, need not be perfectly identical to inhibit expression. Further, the antisense product may hybridize to the untranslated region instead of or in addition to the coding sequence of the gene. The vectors
20 of the present invention can be designed such that the inhibitory effect applies to other proteins within a family of genes exhibiting homology or substantial homology to the target gene.

For antisense suppression, the introduced antisense segment sequence also need not be full length relative to either the primary transcription product or the fully processed mRNA. Generally, a higher percentage of sequence identity can be used to compensate for
25 the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of non-coding segments may be equally effective. Normally, a sequence of between about 30 or 40 nucleotides and the full length of the transcript can be used, though a sequence of at least about 100 nucleotides is preferred, a sequence of at least about 200 nucleotides is more preferred, and a sequence of at least about
30 500 nucleotides is especially preferred.

C.2. Ribozymes

It is also contemplated that gene constructs representing ribozymes and based on the SDFs in TABLE 1 are an object of the invention. Ribozymes can also be used to inhibit expression of genes by suppressing the translation of the mRNA into a polypeptide. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs.

A number of classes of ribozymes have been identified. One class of ribozymes is derived from a number of small circular RNAs, which are capable of self-cleavage and replication in plants. The RNAs replicate either alone (viroid RNAs) or with a helper virus (satellite RNAs). Examples include RNAs from avocado sunblotch viroid and the satellite RNAs from tobacco ringspot virus, lucerne transient streak virus, velvet tobacco mottle virus, solanum nodiflorum mottle virus and subterranean clover mottle virus. The design and use of target RNA-specific ribozymes is described in Haseloff et al. *Nature*, 334:585 (1988).

Like the antisense constructs above, the ribozyme sequence fragment necessary for pairing need not be identical to the target nucleotides to be cleaved, nor identical to the sequences in TABLE 1. Ribozymes may be constructed by combining the ribozyme sequence and some fragment of the target gene which would allow recognition of the target gene mRNA by the resulting ribozyme molecule. Generally, the sequence in the ribozyme capable of binding to the target sequence exhibits a percentage of sequence identity with at least 80%, preferably with at least 85%, more preferably with at least 90% and most preferably with at least 95%, even more preferably, with at least 96%, 97%, 98% or 99% sequence identity to some fragment of a sequence in TABLE 1 or the complement thereof. The ribozyme can be equally effective in inhibiting mRNA translation by cleaving either in the untranslated or coding regions. Generally, a higher percentage of sequence identity can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of non-coding segments may be equally effective.

C.3. Chimeraplasts

The SDFs of the invention, such as those described by Table 1, can also be used to construct chimeraplasts that can be introduced into a cell to produce at least one specific

nucleotide change in a sequence corresponding to the SDF of the invention. A chimeraplast is an oligonucleotide comprising DNA and/or RNA that specifically hybridizes to a target region in a manner which creates a mismatched base-pair. This mismatched base-pair signals the cell's repair enzyme machinery which acts on the mismatched region resulting in the replacement, insertion or deletion of designated nucleotide(s). The altered sequence is then expressed by the cell's normal cellular mechanisms. Chimeraplasts can be designed to repair mutant genes, modify genes, introduce site-specific mutations, and/or act to interrupt or alter normal gene function (US Pat. Nos. 6,010,907 and 6,004,804; and PCT Pub. No. WO99/58723 and WO99/07865).

C.4. Sense Suppression

The SDFs of Table 1 of the present invention are also useful to modulate gene expression by sense suppression. Sense suppression represents another method of gene suppression by introducing at least one exogenous copy or fragment of the endogenous sequence to be suppressed.

Introduction of expression cassettes in which a nucleic acid is configured in the sense orientation with respect to the promoter into the chromosome of a plant or by a self-replicating virus has been shown to be an effective means by which to induce degradation of mRNAs of target genes. For an example of the use of this method to modulate expression of endogenous genes see, Napoli et al., *The Plant Cell* 2:279 (1990), and U.S. Patents Nos. 5,034,323, 5,231,020, and 5,283,184. Inhibition of expression may require some transcription of the introduced sequence.

For sense suppression, the introduced sequence generally will be substantially identical to the endogenous sequence intended to be inactivated. The minimal percentage of sequence identity will typically be greater than about 65%, but a higher percentage of sequence identity might exert a more effective reduction in the level of normal gene products. Sequence identity of more than about 80% is preferred, though about 95% to absolute identity would be most preferred. As with antisense regulation, the effect would likely apply to any other proteins within a similar family of genes exhibiting homology or substantial homology to the suppressing sequence.

C.5. Transcriptional Silencing

The nucleic acid sequences of the invention, including the SDFs of Table 1, and fragments thereof, contain sequences that can be inserted into the genome of an organism

resulting in transcriptional silencing. Such regulatory sequences need not be operatively linked to coding sequences to modulate transcription of a gene. Specifically, a promoter sequence without any other element of a gene can be introduced into a genome to transcriptionally silence an endogenous gene (see, for example, Vaucheret, H et al. (1998) *The Plant Journal* 16: 651-659). As another example, triple helices can be formed using oligonucleotides based on sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto. The oligonucleotide can be delivered to the host cell and can bind to the promoter in the genome to form a triple helix and prevent transcription. An oligonucleotide of interest is one that can bind to the promoter and block binding of a transcription factor to the promoter. In such a case, the oligonucleotide can be complementary to the sequences of the promoter that interact with transcription binding factors.

C.6. Other Methods to Inhibit Gene Expression

Yet another means of suppressing gene expression is to insert a polynucleotide into the gene of interest to disrupt transcription or translation of the gene.

Low frequency homologous recombination can be used to target a polynucleotide insert to a gene by flanking the polynucleotide insert with sequences that are substantially similar to the gene to be disrupted. Sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto can be used for homologous recombination.

In addition, random insertion of polynucleotides into a host cell genome can also be used to disrupt the gene of interest. Azpiroz-Leehan et al., *Trends in Genetics* 13:152 (1997). In this method, screening for clones from a library containing random insertions is preferred to identifying those that have polynucleotides inserted into the gene of interest. Such screening can be performed using probes and/or primers described above based on sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto. The screening can also be performed by selecting clones or R_1 plants having a desired phenotype.

I.D. Methods of Functional Analysis

The constructs described in the methods under I.C. above can be used to determine the function of the polypeptide encoded by the gene that is targeted by the constructs.

Down-regulating the transcription and translation of the targeted gene in the host cell or organisms, such as a plant, may produce phenotypic changes as compared to a wild-type

cell or organism. In addition, *in vitro* assays can be used to determine if any biological activity, such as calcium flux, DNA transcription, nucleotide incorporation, etc., are being modulated by the down-regulation of the targeted gene.

Coordinated regulation of sets of genes, e.g., those contributing to a desired polygenic trait, is sometimes necessary to obtain a desired phenotype. SDFs of the invention representing transcription activation and DNA binding domains can be assembled into hybrid transcriptional activators. These hybrid transcriptional activators can be used with their corresponding DNA elements (i.e., those bound by the DNA-binding SDFs) to effect coordinated expression of desired genes (J.J. Schwarz et al., *Mol. Cell. Biol.* 12:266 (1992), A. Martinez et al., *Mol. Gen. Genet.* 261:546 (1999)).

The SDFs of the invention can also be used in the two-hybrid genetic systems to identify networks of protein-protein interactions (L. McAlister-Henn et al., *Methods* 19:330 (1999), J.C. Hu et al., *Methods* 20:80 (2000), M. Golovkin et al., *J. Biol. Chem.* 274:36428 (1999), K. Ichimura et al., *Biochem. Biophys. Res. Comm.* 253:532 (1998)). The SDFs of the invention can also be used in various expression display methods to identify important protein-DNA interactions (e.g. B. Luo et al., *J. Mol. Biol.* 266:479 (1997)).

I.E. Promoters

The SDFs of the invention are also useful as structural or regulatory sequences in a construct for modulating the expression of the corresponding gene in a plant or other organism, e.g. a symbiotic bacterium. For example, promoter sequences associated to SDFs of Table 1 of the present invention can be useful in directing expression of coding sequences either as constitutive promoters or to direct expression in particular cell types, tissues, or organs or in response to environmental stimuli.

With respect to the SDFs of the present invention a promoter is likely to be a relatively small portion of a genomic DNA (gDNA) sequence located in the first 2000 nucleotides upstream from an initial exon identified in a gDNA sequence or initial ATG or methionine codon or translational start site in a corresponding cDNA sequence. Such promoters are more likely to be found in the first 1000 nucleotides upstream of an initial ATG or methionine codon or translational start site of a cDNA sequence corresponding to a gDNA sequence. In particular, the promoter is usually located upstream of the transcription start site. The fragments of a particular gDNA sequence that function as elements of a promoter in a plant cell will preferably

be found to hybridize to gDNA sequences presented and described in Table 1 at medium or high stringency, relevant to the length of the probe and its base composition.

Promoters are generally modular in nature. Promoters can consist of a basal promoter that functions as a site for assembly of a transcription complex comprising an RNA polymerase, for example RNA polymerase II. A typical transcription complex will include additional factors such as TF_{II}B, TF_{II}D, and TF_{II}E. Of these, TF_{II}D appears to be the only one to bind DNA directly. The promoter might also contain one or more enhancers and/or suppressors that function as binding sites for additional transcription factors that have the function of modulating the level of transcription with respect to tissue specificity and of transcriptional responses to particular environmental or nutritional factors, and the like.

Short DNA sequences representing binding sites for proteins can be separated from each other by intervening sequences of varying length. For example, within a particular functional module, protein binding sites may be constituted by regions of 5 to 60, preferably 10 to 30, more preferably 10 to 20 nucleotides. Within such binding sites, there are typically 2 to 6 nucleotides that specifically contact amino acids of the nucleic acid binding protein. The protein binding sites are usually separated from each other by 10 to several hundred nucleotides, typically by 15 to 150 nucleotides, often by 20 to 50 nucleotides. DNA binding sites in promoter elements often display dyad symmetry in their sequence. Often elements binding several different proteins, and/or a plurality of sites that bind the same protein, will be combined in a region of 50 to 1,000 basepairs.

Elements that have transcription regulatory function can be isolated from their corresponding endogenous gene, or the desired sequence can be synthesized, and recombined in constructs to direct expression of a coding region of a gene in a desired tissue-specific, temporal-specific or other desired manner of inducibility or suppression. When hybridizations are performed to identify or isolate elements of a promoter by hybridization to the long sequences presented in TABLE 1, conditions are adjusted to account for the above-described nature of promoters. For example short probes, constituting the element sought, are preferably used under low temperature and/or high salt conditions. When long probes, which might include several promoter elements are used, low to medium stringency conditions are preferred when hybridizing to promoters across species.

If a nucleotide sequence of an SDF, or part of the SDF, functions as a promoter or fragment of a promoter, then nucleotide substitutions, insertions or deletions that do not substantially affect the binding of relevant DNA binding proteins would be considered equivalent to the exemplified nucleotide sequence. It is envisioned that there are instances

where it is desirable to decrease the binding of relevant DNA binding proteins to silence or down-regulate a promoter, or conversely to increase the binding of relevant DNA binding proteins to enhance or up-regulate a promoter and vice versa. In such instances, polynucleotides representing changes to the nucleotide sequence of the DNA-protein contact region by insertion of additional nucleotides, changes to identity of relevant nucleotides, including use of chemically-modified bases, or deletion of one or more nucleotides are considered encompassed by the present invention. In addition, fragments of the promoter sequences described by Table 1 and variants thereof can be fused with other promoters or fragments to facilitate transcription and/or transcription in specific type of cells or under specific conditions.

Promoter function can be assayed by methods known in the art, preferably by measuring activity of a reporter gene operatively linked to the sequence being tested for promoter function. Examples of reporter genes include those encoding luciferase, green fluorescent protein, GUS, neo, cat and bar.

I.F. UTRs and Junctions

Polynucleotides comprising untranslated (UTR) sequences and intron/exon junctions are also within the scope of the invention. UTR sequences include introns and 5' or 3' untranslated regions (5' UTRs or 3' UTRs). Fragments of the sequences shown in TABLE 1 can comprise UTRs and intron/exon junctions.

These fragments of SDFs, especially UTRs, can have regulatory functions related to, for example, translation rate and mRNA stability. Thus, these fragments of SDFs can be isolated for use as elements of gene constructs for regulated production of polynucleotides encoding desired polypeptides.

Introns of genomic DNA segments might also have regulatory functions. Sometimes regulatory elements, especially transcription enhancer or suppressor elements, are found within introns. Also, elements related to stability of heteronuclear RNA and efficiency of splicing and of transport to the cytoplasm for translation can be found in intron elements. Thus, these segments can also find use as elements of expression vectors intended for use to transform plants.

Just as with promoters UTR sequences and intron/exon junctions can vary from those shown in TABLE 1. Such changes from those sequences preferably will not affect the regulatory activity of the UTRs or intron/exon junction sequences on expression, transcription, or translation unless selected to do so. However, in some instances, down- or

up-regulation of such activity may be desired to modulate traits or phenotypic or *in vitro* activity.

I.G. Coding Sequences

Isolated polynucleotides of the invention can include coding sequences that encode
5 polypeptides comprising an amino acid sequence encoded by sequences in TABLE 1 or an amino acid sequence presented in TABLE 1.

A nucleotide sequence encodes a polypeptide if a cell (or a cell free *in vitro* system) expressing that nucleotide sequence produces a polypeptide having the recited amino acid sequence when the nucleotide sequence is transcribed and the primary transcript is
10 subsequently processed and translated by a host cell (or a cell free *in vitro* system) harboring the nucleic acid. Thus, an isolated nucleic acid that encodes a particular amino acid sequence can be a genomic sequence comprising exons and introns or a cDNA sequence that represents the product of splicing thereof. An isolated nucleic acid encoding an amino acid sequence also encompasses heteronuclear RNA, which contains sequences that are spliced out during
15 expression, and mRNA, which lacks those sequences.

Coding sequences can be constructed using chemical synthesis techniques or by isolating coding sequences or by modifying such synthesized or isolated coding sequences as described above.

In addition to coding sequences encoding the polypeptide sequences of TABLE 1,
20 which are native to corn, *Arabidopsis*, soybean, rice, wheat, and other plants the isolated polynucleotides can be polynucleotides that encode variants, fragments, and fusions of those native proteins. Such polypeptides are described below in part II.

In variant polynucleotides generally, the number of substitutions, deletions or insertions is preferably less than 20%, more preferably less than 15%; even more preferably less than 10%,
25 5%, 3% or 1% of the number of nucleotides comprising a particularly exemplified sequence. It is generally expected that non-degenerate nucleotide sequence changes that result in 1 to 10, more preferably 1 to 5 and most preferably 1 to 3 amino acid insertions, deletions or substitutions will not greatly affect the function of an encoded polypeptide. The most preferred embodiments are those wherein 1 to 20, preferably 1 to 10, most preferably 1 to 5 nucleotides
30 are added to, deleted from and/or substituted in the sequences specifically disclosed in TABLE 1.

Insertions or deletions in polynucleotides intended to be used for encoding a polypeptide preferably preserve the reading frame. This consideration is not so important in instances when the polynucleotide is intended to be used as a hybridization probe.

II. Polypeptides and Proteins

5 IIA. Native polypeptides and proteins

Polypeptides within the scope of the invention include both native proteins as well as variants, fragments, and fusions thereof. Polypeptides of the invention are those encoded by any of the six reading frames of sequences shown in TABLE I, preferably encoded by the
10 three frames reading in the 5' to 3' direction of the sequences as shown.

Native polypeptides include the proteins encoded by the sequences shown in TABLE

1. Such native polypeptides include those encoded by allelic variants.

Polypeptide and protein variants will exhibit at least 75% sequence identity to those
native polypeptides of TABLE I. More preferably, the polypeptide variants will exhibit at least
15 85% sequence identity; even more preferably, at least 90% sequence identity; more preferably at
least 95%, 96%, 97%, 98%, or 99% sequence identity. Fragments of polypeptide or fragments
of polypeptides will exhibit similar percentages of sequence identity to the relevant fragments
of the native polypeptide. Fusions will exhibit a similar percentage of sequence identity in that
fragment of the fusion represented by the variant of the native peptide.

20 Furthermore, polypeptide variants will exhibit at least one of the functional properties of
the native protein. Such properties include, without limitation, protein interaction, DNA
interaction, biological activity, immunological activity, receptor binding, signal transduction,
transcription activity, growth factor activity, secondary structure, three-dimensional structure,
etc. As to properties related to *in vitro* or *in vivo* activities, the variants preferably exhibit at least
25 60% of the activity of the native protein; more preferably at least 70%, even more preferably at
least 80%, 85%, 90% or 95% of at least one activity of the native protein.

One type of variant of native polypeptides comprises amino acid substitutions, deletions
and/or insertions. Conservative substitutions are preferred to maintain the function or activity of
the polypeptide.

30 Within the scope of percentage of sequence identity described above, a polypeptide of
the invention may have additional individual amino acids or amino acid sequences inserted into
the polypeptide in the middle thereof and/or at the N-terminal and/or C-terminal ends thereof.

Likewise, some of the amino acids or amino acid sequences may be deleted from the polypeptide.

A.1 Antibodies

Isolated polypeptides can be utilized to produce antibodies. Polypeptides of the invention can generally be used, for example, as antigens for raising antibodies by known techniques. The resulting antibodies are useful as reagents for determining the distribution of the antigen protein within the tissues of a plant or within a cell of a plant. The antibodies are also useful for examining the production level of proteins in various tissues, for example in a wild-type plant or following genetic manipulation of a plant, by methods such as Western blotting.

Antibodies of the present invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the polypeptides of the invention are first used to immunize a suitable animal, such as a mouse, rat, rabbit, or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies as detection reagents. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by *in vitro* immunization using methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization.

Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating the blood at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000xg for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the method of Kohler and Milstein, *Nature* 256: 495 (1975), or modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells can be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate, or well, coated with the protein antigen. B-cells producing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with

the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, HAT[®]). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected Mab-secreting hybridomas are then cultured either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

Other methods for sustaining antibody-producing B-cell clones, such as by EBV transformation, are known.

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ³²P and ¹²⁵I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TNB) to a blue pigment, quantifiable with a spectrophotometer.

A.2 In Vitro Applications of Polypeptides

Some polypeptides of the invention will have enzymatic activities that are useful *in vitro*. For example, the soybean trypsin inhibitor (Kunitz) family is one of the numerous families of proteinase inhibitors. It comprises plant proteins which have inhibitory activity against serine proteinases from the trypsin and subtilisin families, thiol proteinases and aspartic proteinases. Thus, these peptides find *in vitro* use in protein purification protocols and perhaps in therapeutic settings requiring topical application of protease inhibitors.

Delta-aminolevulinic acid dehydratase (EC 4.2.1.24) (ALAD) catalyzes the second step in the biosynthesis of heme, the condensation of two molecules of 5-aminolevulinate to form porphobilinogen and is also involved in chlorophyll biosynthesis (Kaczor et al. (1994) Plant Physiol. 1-4: 1411-7; Smith (1988) Biochem. J. 249: 423-8; Schneider (1976) Z. naturforsch. [C] 31: 55-63). Thus, ALAD proteins can be used as catalysts in synthesis of heme derivatives. Enzymes of biosynthetic pathways generally can be used as catalysts for *in vitro* synthesis of the compounds representing products of the pathway.

Polypeptides encoded by SDFs of the invention can be engineered to provide purification reagents to identify and purify additional polypeptides that bind to them. This allows one to identify proteins that function as multimers or elucidate signal transduction or

metabolic pathways. In the case of DNA binding proteins, the polypeptide can be used in a similar manner to identify the DNA determinants of specific binding (S. Pierrou et al., *Anal. Biochem.* 229:99 (1995), S. Chusacultachai et al., *J. Biol. Chem.* 274:23591 (1999), Q. Lin et al., *J. Biol. Chem.* 272:27274 (1997)).

5 II.B. POLYPEPTIDE VARIANTS, FRAGMENTS, AND FUSIONS

Generally, variants, fragments, or fusions of the polypeptides encoded by the SDFs of the invention can exhibit at least one of the activities of the identified domains and/or related polypeptides described in Table 1 corresponding to the SDF of interest.

II.B.(1) Variants

- 10 A type of variant of the native polypeptides comprises amino acid substitutions. Conservative substitutions, described above (see II.), are preferred to maintain the function or activity of the polypeptide. Such substitutions include conservation of charge, polarity, hydrophobicity, size, etc. For example, one or more amino acid residues within the sequence can be substituted with another amino acid of similar polarity that acts as a functional
- 15 equivalent, for example providing a hydrogen bond in an enzymatic catalysis. Substitutes for an amino acid within an exemplified sequence are preferably made among the members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and
- 20 glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

- Within the scope of percentage of sequence identity described above, a polypeptide of the invention may have additional individual amino acids or amino acid sequences inserted into the polypeptide in the middle thereof and/or at the N-terminal and/or C-terminal ends thereof.
- 25 Likewise, some of the amino acids or amino acid sequences may be deleted from the polypeptide. Amino acid substitutions may also be made in the sequences; conservative substitutions being preferred.

- One preferred class of variants are those that comprise (1) the domain of an encoded polypeptide and/or (2) residues conserved between the encoded polypeptide and
- 30 related polypeptides. For this class of variants, the encoded polypeptide sequence is changed by insertion, deletion, or substitution at positions flanking the domain and/or conserved residues.

Another class of variants includes those that comprise an encoded polypeptide sequence that is changed in the domain or conserved residues by a conservative substitution.

Yet another class of variants includes those that lack one of the *in vitro* activities, or structural features of the encoded polypeptides. One example is polypeptides or proteins produced from genes comprising dominant negative mutations. Such a variant may comprise an encoded polypeptide sequence with non-conservative changes in a particular domain or group of conserved residues.

II.A.(2) FRAGMENTS

Fragments of particular interest are those that comprise a domain identified for a polypeptide encoded by an SDF of the instant invention and variants thereof. Also, fragments that comprise at least one region of residues conserved between an SDF encoded polypeptide and its related polypeptides are of great interest. Fragments are sometimes useful as polypeptides corresponding to genes comprising dominant negative mutations are.

II.A.(3) FUSIONS

Of interest are chimeras comprising (1) a fragment of the SDF encoded polypeptide or variants thereof of interest and (2) a fragment of a polypeptide comprising the same domain. For example, an AP2 helix encoded by a SDF of the invention fused to second AP2 helix from ANT protein, which comprises two AP2 helices. The present invention also encompasses fusions of SDF encoded polypeptides, variants, or fragments thereof fused with related proteins or fragments thereof.

DEFINITION OF DOMAINS

The polypeptides of the invention may possess identifying domains. In addition, the domains within the SDF encoded polypeptide can be defined by the region that exhibits at least 70% sequence identity with the consensus sequences listed in the detailed description below of each of the domains.

The majority of the protein domain descriptions given below are obtained from Prosite, (<http://www.expasy.ch/prosite/>), and Pfam, (<http://pfam.wustl.edu/browse.shtml>).

1. (AAA) AAA-protein family signature

- A large family of ATPases has been described [1 to 5] whose key feature is that they share a conserved region of about 220 amino acids that contains an ATP-binding site. This family is now called AAA, for 'ATPases 'A'associated with diverse cellular 'A'ctivities. The proteins that belong to this family either contain one or two AAA domains. Proteins containing two AAA domains:
- Mammalian and drosophila NSF (N-ethylmaleimide-sensitive fusion protein) and the fungal homolog, SEC18. These proteins are involved in intracellular transport between the endoplasmic reticulum and Golgi, as well as between different Golgi cisternae.
 - Mammalian transitional endoplasmic reticulum ATPase (previously known as p97 or VCP) which is involved in the transfer of membranes from the endoplasmic reticulum to the golgi apparatus. This protein forms a ring-shaped homooligomer composed of six subunits. The yeast homolog is CDC48 and it may play a role in spindle pole proliferation.
 - Yeast protein PAS1, essential for peroxisome assembly and the related protein PAS1 from *Pichia pastoris*.
 - Yeast protein AFG2.
 - *Sulfolobus acidocaldarius* protein SAV and *Halobacterium salinarum* cdcH which may be part of a transduction pathway connecting light to cell division.
- Proteins containing a single AAA domain:
- *Escherichia coli* and other bacteria ftsH (or hflB) protein. FtsH is an ATP-dependent zinc metallopeptidase that seems to degrade the heat-shock sigma-32 factor. It is an integral membrane protein with a large cytoplasmic C-terminal domain that contain both the AAA and the protease domains.
 - Yeast protein YME1, a protein important for maintaining the integrity of the mitochondrial compartment. YME1 is also a zinc-dependent protease.
 - Yeast protein AFG3 (or YTA10). This protein also seems to contain a AAA domain followed by a zinc-dependent protease domain.
- Subunits from the regulatory complex of the 26S proteasome [6] which is involved in the ATP-dependent degradation of ubiquitinated proteins:
- a) Mammalian subunit 4 and homologs in other higher eukaryotes, in yeast (gene YTA5) and fission yeast (gene mts2).
 - b) Mammalian subunit 6 (TBP7) and homologs in other higher eukaryotes and in yeast (gene YTA2).

- c) Mammalian subunit 7 (MSS1) and homologs in other higher eukaryotes and in yeast (gene CIM5 or YTA3).
- d) Mammalian subunit 8 (P45) and homologs in other higher eukaryotes and in yeast (SUG1 or CIM3 or TBY1) and fission yeast (gene let1).

5 Other probable subunits such as human TBP1 which seems to influences HIV gene expression by interacting with the virus tat transactivator protein and yeast YTA1 and YTA6.

- Yeast protein BCS1, a mitochondrial protein essential for the expression of the Rieske iron-sulfur protein.
- Yeast protein MSP1, a protein involved in intramitochondrial sorting of proteins.
- 10 - Yeast protein PAS8, and the corresponding proteins PAS5 from *Pichia pastoris* and PAY4 from *Yarrowia lipolytica*.
- Mouse protein SKD1 and its fission yeast homolog (SpAC2G11.06).
- *Caenorhabditis elegans* meiotic spindle formation protein mei-1.
- Yeast protein SAPI.
- 15 - Yeast protein YTA7.
- *Mycobacterium leprae* hypothetical protein A2126A.

It is proposed that, in general, the AAA domains in these proteins act as ATP-dependent protein clamps [5]. In addition to the ATP-binding 'A' and 'B' motifs, which are located in the N-terminal half of this domain, there is a highly conserved region located in the central part of the domain which was used to develop a signature pattern.

Consensus pattern: [LIVMT]-x-[LIVMT]-[LIVMF]-x-[GATMC]-[ST]-[NS]-x(4)-[LIVM]-D-x-A-[LIFA]-x-R

- 25 [1] Froehlich K.-U., Fries H.W., Ruediger M., Erdmann R., Botstein D., Mecke D. J. Cell Biol. 114:443-453(1991).
- [2] Erdmann R., Wiebel F.F., Flessau A., Rytka J., Beyer A., Froehlich K.-U., Kunau W.-H. Cell 64:499-510(1991).
- [3] Peters J.-M., Walsh M.J., Franke W.W. EMBO J. 9:1757-1767(1990).
- 30 [4] Kunau W.-H., Beyer A., Goette K., Marzioch M., Saidowsky J., Skaletz-Rorowski A., Wiebel F.F. Biochimie 75:209-224(1993).
- [5] Confalonieri F., Duguët M. BioEssays 17:639-650(1995).[6] Hilt W., Wolf D.H. Trends Biochem. Sci. 21:96-102(1996).

2. ABC Membrane (ABC transporter transmembrane region). This family represents a unit of six transmembrane helices. Many members of the ABC transporter family (ABC_tran) have two such regions. See also descriptions of ABC Tran, below, and ABC2 membrane, above.

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3. (ABC Tran) ABC transporters family signature. On the basis of sequence similarities a family of related ATP-binding proteins has been characterized [1 to 5]. These proteins are associated with a variety of distinct biological processes in both prokaryotes and eukaryotes, but a majority of them are involved in active transport of small hydrophilic molecules across the cytoplasmic membrane. All these proteins share a conserved domain of some two hundred amino acid residues, which includes an ATP-binding site. These proteins are collectively known as ABC transporters. Proteins known to belong to this family are listed below (references are only provided for recently determined sequences). In prokaryotes: - Active transport systems components: alkylphosphonate uptake (phnC/phnK/ phnL);
- 15 arabinose (araG); arginine (artP); dipeptide (dcfAD; dppD/dppF); ferric enterobactin (fepC); ferrichrome (fhuC); galactoside (mglA); glutamine (glnQ); glycerol-3-phosphate (ugpC); glycine betaine/L-proline (proV); glutamate/aspartate (gltL); histidine (hisP); iron(III) (sfuC), iron(III) dicitrate (fecE); lactose (lacK); leucine/isoleucine/valine (braF/braG; livF/livG); maltose (malK); molybdenum (modC); nickel (nikD/ nikE); oligopeptide
- 20 (amiE/amiF; oppD/oppF); peptide (sapD/sapF); phosphate (pstB); putrescine (potG); ribose (rbsA); spermidine/putrescine (potA); sulfate (cysA); vitamin B12 (btuD). - Hemolysin/leukotoxin export proteins hlyB, cyaB and lktB. - Colicin V export protein cvaB. - Lactococcal export protein lcnC [6]. - Lantibiotic transport proteins nisT (nisin) and spaT (subtilin). - Extracellular proteases B and C export protein prtD. - Alkaline protease secretion
- 25 protein aprD. - Beta-(1,2)-glucan export proteins chvA and ndvA. - Haemophilus influenzae capsule-polysaccharide export protein bexA. - Cytochrome c biogenesis proteins ccmA (also known as cycV and helA). - Polysialic acid transport protein kpsT. - Cell division associated ftsE protein (function unknown). - Copper processing protein nosF from *Pseudomonas stutzeri*. - Nodulation protein nodJ from *Rhizobium* (function unknown). - *Escherichia coli*
- 30 proteins cydC and cydD. - Subunit A of the ABC excision nuclease (gene *uvrA*). - Erythromycin resistance protein from *Staphylococcus epidermidis* (gene *msrA*). - Tylosin resistance protein from *Streptomyces fradiae* (gene *tlrC*) [7]. - Heterocyst differentiation protein (gene *hetA*) from *Anabaena* PCC 7120. - Protein P29 from *Mycoplasma hyorhinis*, a probable component of a high affinity transport system. - yhbG, a putative protein whose

gene is linked with *ntrA* in many bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas putida*, *Rhizobium meliloti* and *Thiobacillus ferrooxidans*. - *Escherichia coli* and related bacteria hypothetical proteins *yabJ*, *yadG*, *yagC*, *ybbA*, *ycjW*, *yddA*, *yehX*, *yejF*, *yheS*, *yhiG*, *yhiH*, *yjcW*, *yjjK*, *yoiJ*, *yrbF* and *ytfR*. In eukaryotes: - The multidrug

5 transporters (Mdr) (P-glycoprotein), a family of closely related proteins which extrude a wide variety of drugs out of the cell (for a review see [8]). - Cystic fibrosis transmembrane conductance regulator (CFTR), which is most probably involved in the transport of chloride ions. - Antigen peptide transporters 1 (TAP1, PSF1, RING4, HAM-1, mtp1) and 2 (TAP2, PSF2, RING11, HAM-2, mtp2), which are involved in the transport of antigens from the

10 cytoplasm to a membrane-bound compartment for association with MHC class I molecules. - 70 Kd peroxisomal membrane protein (PMP70). - ALDP, a peroxisomal protein involved in X-linked adrenoleukodystrophy [9]. - Sulfonylurea receptor [10], a putative subunit of the B-cell ATP-sensitive potassium channel. - *Drosophila* proteins white (w) and brown (bw), which are involved in the import of ommatidium screening pigments. - Fungal elongation

15 factor 3 (EF-3). - Yeast STE6 which is responsible for the export of the α -factor pheromone. - Yeast mitochondrial transporter ATM1. - Yeast MDL1 and MDL2. - Yeast SNQ2. - Yeast sporidesmin resistance protein (gene *PDR5* or *STS1* or *YDR1*). - Fission yeast heavy metal tolerance protein *hmt1*. This protein is probably involved in the transport of metal-bound phytochelatin. - Fission yeast brefeldin A resistance protein (gene *bfr1* or *hba2*). - Fission

20 yeast leptomycin B resistance protein (gene *pmd1*). - *mbpX*, a hypothetical chloroplast protein from Liverwort. - Prestalk-specific protein *tagB* from slime mold. This protein consists of two domains: a N-terminal subtilase catalytic domain and a C-terminal ABC transporter domain. As a signature pattern for this class of proteins, a conserved region which is located between the 'A' and the 'B' motifs of the ATP-binding site was used.

25 Consensus pattern: [LIVMFYC]-[SA]-[SAPGLVFYKOH]-G-[DENQMW]-[KRQASPCLIMFW]-[KRNQSTAVM]-[KRACLVM]-[LIVMFYPAN]-{PHY}-[LIVMFW]-[SAGCLIVP]-{FYWHP}-[KRHP]-[LIVMFYWSTA] The ATP-binding region is duplicated in *araG*, *mdl*, *msrA*, *rbsA*, *tlrC*, *uvrA*, *yejF*, Mdr's, CFTR, *pmd1* and in EF-3. In

30 some of those proteins, the above pattern only detect one of the two copies of the domain. The proteins belonging to this family also contain one or two copies of the ATP-binding motifs 'A' and 'B'.

- [1] Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P. J. Bioenerg. Biomembr. 22:571-592(1990).
- [2] Higgins C.F., Gallagher M.P., Mimmack M.M., Pearce S.R. BioEssays 8:111-116(1988).
- [3] Higgins C.F., Hiles I.D., Salmond G.P.C., Gill D.R., Downie J.A., Evans I.J., Holland I.B., Gray L., Buckels S.D., Bell A.W., Hermodson M.A. Nature 323:448-450(1986).
- [4] Doolittle R.F., Johnson M.S., Husain I., van Houten B., Thomas D.C., Sancar A. Nature 323:451-453(1986).
- [5] Blight M.A., Holland I.B. Mol. Microbiol. 4:873-880(1990).
- [6] Stoddard G.W., Petzel J.P., van Belkum M.J., Kok J., McKay L.L. Appl. Environ. Microbiol. 58:1952-1961(1992).
- [7] Rosteck P.R. Jr., Reynolds P.A., Hershberger C.L. Gene 102:27-32(1991).
- [8] Gottesman M.M., Pastan I. J. Biol. Chem. 263:12163-12166(1988).
- [9] Valle D., Gaertner J. Nature 361:682-683(1993).
- [10] Aguilar-Bryan L., Nichols C.G., Wechsler S.W., Clement J.P. IV, Boyd A.E. III, Gonzalez G., Herrera-Sosa H., Nguy K., Bryan J., Nelson D.A. Science 268:423-426(1995).

4. (ACBP)

Acyl-CoA-binding protein signature

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Acyl-CoA-binding protein (ACBP) is a small (10 Kd) protein that binds medium- and long-chain acyl-CoA esters with very high affinity and may function as an intracellular carrier of acyl-CoA esters [1]. ACBP is also known as diazepam binding inhibitor (DBI) or endozepine (EP) because of its ability to displace diazepam from the benzodiazepine (BZD) recognition site located on the GABA type A receptor. It is therefore possible that this protein also acts as a neuropeptide to modulate the action of the GABA receptor [2]. ACBP is a highly conserved protein of about 90 residues that has been so far found in vertebrates, insects and yeast. ACBP is also related to the N-terminal section of a probable transmembrane protein of unknown function which has been found in mammals. As a signature pattern, the region that corresponds to residues 19 to 37 in mammalian ACBP was selected.

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Consensus pattern: P-[STA]-x-[DEN]-x-[LIVMF]-x(2)-[LIVMFY]-Y-[GSTA]-x-[FY]-K- Q-[STA](2)-x-G-

- [1] Rose T.M., Schultz E.R., Todaro G.J. Proc. Natl. Acad. Sci. U.S.A. 89:11287-11291(1992).
 [2] Costa E., Guidotti A. Life Sci. 49:325-344(1991).

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5. (AIRS)

AIR synthase related proteins

This family includes Hydrogen expression/formation protein HypE, AIR synthases, FGAM synthase and selenide, water dikinase.

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6. (AMP-binding)

Putative AMP-binding domain signature

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It has been shown [1 to 5] that a number of prokaryotic and eukaryotic enzymes which all probably act via an ATP-dependent covalent binding of AMP to their substrate, share a region of sequence similarity. These enzymes are: - Insects luciferase (luciferin 4-monooxygenase). Luciferase produces light by catalyzing the oxidation of luciferin in presence of ATP and molecular oxygen. - Alpha-aminoadipate reductase from yeast (gene LYS2). This enzyme catalyzes the activation of alpha-aminoadipate by ATP-dependent adenylation and the reduction of activated alpha-aminoadipate by NADPH. - Acetate--CoA ligase (acetyl-CoA synthetase), an enzyme that catalyzes the formation of acetyl-CoA from acetate and CoA. - Long-chain-fatty-acid--CoA ligase, an enzyme that activates long-chain fatty acids for both the synthesis of cellular lipids and their degradation via beta-oxidation. - 4-coumarate--CoA ligase (4CL), a plant enzyme that catalyzes the formation of 4-coumarate-CoA from 4-coumarate and coenzyme A; the branchpoint reactions between general phenylpropanoid metabolism and pathways leading to various specific end products. - O-succinylbenzoic acid--CoA ligase (OSB-CoA synthetase) (gene menE) [6], a bacterial enzyme involved in the biosynthesis of menaquinone (vitamin K2). - 4-Chlorobenzoate--CoA ligase (EC 6.2.1.-) (4-CBA--CoA ligase) [7], a Pseudomonas enzyme involved in the degradation of 4-CBA. - Indoleacetate-lysine ligase (IAA-lysine synthetase) [8], an enzyme from Pseudomonas syringae that converts indoleacetate to IAA-lysine. - Bile acid-CoA ligase (gene baiB) from Eubacterium strain VPI 12708 [4]. This enzyme catalyzes the ATP-

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- dependent formation of a variety of C-24 bile acid-CoA. - Crotonobetaine/carnitine-CoA ligase (EC 6.3.2.-) from *Escherichia coli* (gene *caiC*). - L-(alpha-aminoadipyl)-L-cysteinyl-D-valine synthetase (ACV synthetase) from various fungi (gene *acvA* or *pcbAB*). This enzyme catalyzes the first step in the biosynthesis of penicillin and cephalosporin, the formation of
- 5 ACV from the constituent amino acids. The amino acids seem to be activated by adenylation. It is a protein of around 3700 amino acids that contains three related domains of about 1000 amino acids. - Gramicidin S synthetase I (gene *grsA*) from *Bacillus brevis*. This enzyme catalyzes the first step in the biosynthesis of the cyclic antibiotic gramicidin S, the ATP-dependent racemization of phenylalanine - Tyrocidine synthetase I (gene *tycA*) from
 - 10 *Bacillus brevis*. The reaction carried out by *tycA* is identical to that catalyzed by *grsA* - Gramicidin S synthetase II (gene *grsB*) from *Bacillus brevis*. This enzyme is a multifunctional protein that activates and polymerizes proline, valine, ornithine and leucine. *GrsB* consists of four related domains. - Enterobactin synthetase components E (gene *entE*) and F (gene *entF*) from *Escherichia coli*. These two enzymes are involved in the ATP-
 - 15 dependent activation of respectively 2,3-dihydroxybenzoate and serine during enterobactin (enterochelin) biosynthesis. - Cyclic peptide antibiotic surfactin synthase subunits 1, 2 and 3 from *Bacillus subtilis*. Subunits 1 and 2 contains three related domains while subunit 3 only contains a single domain. - HC-toxin synthetase (gene *HTS1*) from *Cochliobolus carbonum*. This enzyme activates the four amino acids (Pro, L-Ala, D-Ala and 2-amino-9,10-epoxy-8-
 - 20 oxodecanoic acid) that make up HC-toxin, a cyclic tetrapeptide. *HTS1* consists of four related domains. There are also some proteins, whose exact function is not yet known, but which are, very probably, also AMP-binding enzymes. These proteins are: - *ORA* (octapeptide-repeat antigen), a *Plasmodium falciparum* protein whose function is not known but which shows a high degree of similarity with the above proteins. - *AngR*, a *Vibrio anguillarum* protein.
 - 25 *AngR* is thought to be a transcriptional activator which modulates the anguibactin (an iron-binding siderophore) biosynthesis gene cluster operon. But it is believed [9], that *angR* is not a DNA-binding protein, but rather an enzyme involved in the biosynthesis of anguibactin. This conclusion is based on three facts: the presence of the AMP-binding domain; the size of *angR* (1048 residues), which is far bigger than any bacterial transcriptional protein; and the
 - 30 presence of a probable S-acyl thioesterase immediately downstream of *angR*. - A hypothetical protein in *mmsB* 3' region in *Pseudomonas aeruginosa*. - *Escherichia coli* hypothetical protein *ydiD*. - Yeast hypothetical protein *YBR041w*. - Yeast hypothetical protein *YBR222c*. - Yeast hypothetical protein *YER147c*. All these proteins contain a highly conserved region very rich in glycine, serine, and threonine which is followed by a conserved

lysine. A parallel can be drawn between this type of domain and the G-x(4)-G-K-[ST] ATP-/GTP-binding 'P-loop' domain or the protein kinases G-x-G-x(2)-[SG]-x(10,20)-KATP-binding domains.

- 5 Consensus pattern: [LIVMFY]-x(2)-[STG]-[STAG]-G-[ST]-[STEI]-[SG]-x-[PASLIVM]-[KR] In a majority of cases the residue that follows the Lys at the end of the pattern is a Gly.

[1] Toh H. Protein Seq. Data Anal. 4:111-117(1991).

[2] Smith D.J., Earl A.J., Turner G. EMBO J. 9:2743-2750(1990).

- 10 [3] Schroeder J. Nucleic Acids Res. 17:460-460(1989).

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[7] Babbitt P.C., Kenyon G.L., Matin B.M., Charest H., Sylvestre M., Scholten J.D., Chang

- 15 K.-H., Liang P.-H., Dunaway-Mariano D. Biochemistry 31:5594-5604(1992).

[8] Farrell D.H., Mikesell P., Actis L.A., Crosa J.H. Gene 86:45-51(1990).

7. AP2 domain

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This 60 amino acid residue domain can bind to DNA [1]. This domain is plant specific. Members of this family are suggested to be related to pyridoxal phosphate-binding domains such as found in aminotran_2 [3]. AP2 domains are also described in Jofuku et al., co-pending U.S. Patent applications 08/700,152, 08/879,827, 08/912,272, 09/026,039.

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[1] Ohme-takagi M, Shinshi H: Plant Cell 1995;7:173-182.

[2] Weigel D: Plant Cell 1995;7:388-389.

[3] Mushegian AR, Koonin EV: Genetics 1996;144:817-828.

30

8. ARID

The ARID domain is an AT-Rich Interaction domain sharing structural homology to DNA replication and repair nucleases and polymerases.

- [1] Herrscher RF, Kaplan MH, Lelsz DL, Das C, Scheuermann R, Tucker PW; Genes Dev 1995;9:3067-3082.
- [2] Yuan YC, Whitson RH, Liu Q, Itakura K, Chen Y; Nat Struct Biol 1998;5:959-964.

5

9. (ATP synt)

ATP synthase gamma subunit signature

- ATP synthase (proton-translocating ATPase) (EC 3.6.1.34) [1,2] is a component of the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), and a catalytic core, called coupling factor CF(1). The former acts as a proton channel; the latter is composed of five subunits, alpha, beta, gamma, delta and epsilon. Subunit gamma is believed to be important in regulating ATPase activity and the flow of protons through the CF(0) complex. The best conserved region of the gamma subunit [3] is its C-terminus which seems to be essential for assembly and catalysis. As a signature pattern to detect ATPase gamma subunits, a 14 residue conserved segment where the last amino acid is found one to three residues from the C-terminal extremity was used.
- Consensus pattern: [IV]-T-x-E-x(2)-[DE]-x(3)-G-A-x-[SAKR]- Note: Pea chloroplast gamma and two Bacillus species gamma subunits are not detected by this motif.

- [1] Futai M., Noumi T., Maeda M. Annu. Rev. Biochem. 58:111-136(1989).
- [2] Senior A.E. Physiol. Rev. 68:177-231(1988).
- [3] Miki J., Maeda M., Mukohata Y., Futai M. FEBS Lett. 232:221-226(1988).

30

10. (ATP Synt A)

Synthase a subunit signature

ATP synthase (proton-translocating ATPase) (EC 3.6.1.34) [1,2] is a component of the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), which acts as a proton channel, and a catalytic core,

termed coupling factor CF(1). The CF(0) a subunit, also called protein 6, is a key component of the proton channel; it may play a direct role in translocating protons across the membrane. It is a highly hydrophobic protein that has been predicted to contain 8 transmembrane regions [3]. Sequence comparison of a subunits from all available sources reveals very few conserved regions. The best conserved region is located in what is predicted to be the fifth transmembrane domain. This region contains three perfectly conserved residues: an arginine, a leucine and an asparagine. Mutagenesis experiments of ATPase activity. This region was selected as a signature pattern.

- 10 Consensus pattern: [STAGN]-x-[STAG]-[LIVMF]-R-L-x-[SAGV]-N-[LIVMT] [R is important for proton translocation]

[1] Futai M., Noumi T., Maeda M. Annu. Rev. Biochem. 58:111-136(1989).

[2] Senior A.E. Physiol. Rev. 68:177-231(1988).

- 15 [3] Lewis M.L., Chang J.A., Simoni R.D. J. Biol. Chem. 265:10541-10550(1990).

[4] Cain B.D., Simoni R.D. J. Biol. Chem. 264:3292-3300(1989).

11. ATP synthase B

- 20 Part of the CF(0) (base unit) of the ATP synthase. The base unit is thought to translocate protons through membrane (inner membrane in mitochondria, thylakoid membrane in plants, cytoplasmic membrane in bacteria). The B subunits are thought to interact with the stalk of the CF(1) subunits.

25

12. (ATP synt C)

ATP synthase c subunit signature

- ATP synthase (proton-translocating ATPase) [1,2] is a component of the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), which acts as a proton channel, and a catalytic core, termed coupling factor CF(1). The CF(0) c subunit (also called protein 9, proteolipid, or subunit III) [3,4] is a highly hydrophobic protein of about 8 Kd which has been implicated in the proton-conducting

activity of ATPase. Structurally subunit c consist of two long terminal hydrophobic regions, which probably span the membrane, and a central hydrophilic region. N,N'-dicyclohexylcarbodiimide (DCCD) can bind covalently to subunit c and thereby abolish the ATPase activity. DCCD binds to a specific glutamate or aspartate residue which is located in the middle of the second hydrophobic region near the C-terminus of the protein. A signature pattern which includes the DCCD-binding residue was derived.

Consensus pattern: [GSTA]-R-[NQ]-P-x(10)-[LIVMFYW](2)-x(3)-[LIVMFYW]-x-[DE] [D or E binds DCCD]

[1] Futai M., Noumi T., Maeda M. Annu. Rev. Biochem. 58:111-136(1989).

[2] Senior A.E. Physiol. Rev. 68:177-231(1988).

[3] Ivaschenko A.T., Karpenyuk T.A., Ponomarenko S.V. Biokhimiia 56:406-419(1991).

[4] Recipon H., Perasso R., Adoutte A., Quetier F. J. Mol. Evol. 34:292-303(1992).

13. (ATP synt DE)

ATP synthase, Delta/Epsilon chain

Part of the ATP synthase CF(1). These subunits are part of the head unit of the ATP synthase. The subunits are called delta and epsilon in human and metazoan species but in bacterial species the delta (D) subunit is the equivalent to the Oligomycin sensitive subunit (OSCP) in metazoans.

14. (ATP synt ab)

ATP synthase alpha and beta subunits signature

ATP synthase (proton-translocating ATPase) [1,2] is a component of the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), and a catalytic core, called coupling factor CF(1). The former acts as a proton channel; the latter is composed of five subunits, alpha, beta, gamma, delta and epsilon. The sequences of subunits alpha and beta are related and both contain a nucleotide-binding site

for ATP and ADP. The beta chain has catalytic activity, while the alpha chain is a regulatory subunit. Vacuolar ATPases [3] (V-ATPases) are responsible for acidifying a variety of intracellular compartments in eukaryotic cells. Like F-ATPases, they are oligomeric complexes of a transmembrane and a catalytic sector. The sequence of the largest subunit of the catalytic sector (70 Kd) is related to that of F-ATPase beta subunit, while a 60 Kd subunit, from the same sector, is related to the F-ATPases alpha subunit [4]. Archaeobacterial membrane-associated ATPases are composed of three subunits. The alpha chain is related to F-ATPases beta chain and the beta chain is related to F-ATPases alpha chain [4]. A protein highly similar to F-ATPase beta subunits is found [5] in some bacterial apparatus involved in a specialized protein export pathway that proceeds without signal peptide cleavage. This protein is known as flil in *Bacillus* and *Salmonella*, Spa47 (mxiB) in *Shigella flexneri*, HrpB6 in *Xanthomonas campestris* and yscN in *Yersinia* virulence plasmids. To detect these ATPase subunits, a segment of ten amino-acid residues, containing two conserved serines, as a signature pattern was selected. The first serine seems to be important for catalysis - in the ATPase alpha chain at least - as its mutagenesis causes catalytic impairment.

Consensus pattern: P-[SAP]-[LIV]-[DNH]-x(3)-S-x-S [The first S is a putative active site residue]

- [1] Futai M., Noumi T., Maeda M. *Annu. Rev. Biochem.* 58:111-136(1989).
- [2] Senior A.E. *Physiol. Rev.* 68:177-231(1988).
- [3] Nelson N. J. *Bioenerg. Biomembr.* 21:553-571(1989).
- [4] Gogarten J.P., Kibak H., Dittrich P., Taiz L., Bowman E.J., Bowman B.J., Manolson M.F., Poole R.J., Date T., Oshima T., Konishi J., Denda K., Yoshida M. *Proc. Natl. Acad. Sci. U.S.A.* 86:6661-6665(1989).
- [5] Dreyfus G., Williams A.W., Kawagishi I., MacNab R.M. *J. Bacteriol.* 175:3131-3138(1993).

15. (ATP synt ab C)
ATP synthase ab C terminal.

Number of members: 190

[1] Abrahams JP, Leslie AG, Lutter R, Walker JE; Structure at 2.8 Å resolution of F1-ATPase from bovine heart mitochondria." *Nature* 1994;370:621-628.

5 16. (A deaminase)

Adenosine and AMP deaminase signature

Adenosine deaminase catalyzes the hydrolytic deamination of adenosine into inosine. AMP deaminase catalyzes the hydrolytic deamination of AMP into IMP. It has been shown [1] that
10 these two types of enzymes share three regions of sequence similarities; these regions are centered on residues which are proposed to play an important role in the catalytic mechanism of these two enzymes. One of these regions, containing two conserved aspartic acid residues that are potential active site residues was selected.

15 Consensus pattern: [SA]-[LIVM]-[NGS]-[STA]-D-D-P [The two D's are putative active site residues]

[1] Chang Z., Nygaard P., Chinault A.C., Kellems R.E. *Biochemistry* 30:2273-2280(1991).

20

17. (Acetyltransf)

Acetyltransferase (GNAT) family.

This family contains proteins with N-acetyltransferase functions.

25

[1] Neuwald AF, Landsman D; *Trends Biochem Sci* 1997;22:154-155.

18. (Aconitase C)

30 Aconitase family signature

Aconitase (aconitate hydratase) (EC 4.2.1.3) [1] is the enzyme from the tricarboxylic acid cycle that catalyzes the reversible isomerization of citrate and isocitrate. Cis-aconitate is formed as an intermediary product during the course of the reaction. In eukaryotes two

isozymes of aconitase are known to exist: one found in the mitochondrial matrix and the other found in the cytoplasm. Aconitase, in its active form, contains a 4Fe-4S iron-sulfur cluster; three cysteine residues have been shown to be ligands of the 4Fe-4S cluster. It has been shown that the aconitase family also contains the following proteins: - Iron-responsive element binding protein (IRE-BP). IRE-BP is a cytosolic protein that binds to iron-responsive elements (IREs). IREs are stem-loop structures found in the 5'UTR of ferritin, and delta aminolevulinic acid synthase mRNAs, and in the 3'UTR of transferrin receptor mRNA. IRE-BP also express aconitase activity. - 3-isopropylmalate dehydratase (EC 4.2.1.33) (isopropylmalate isomerase), the enzyme that catalyzes the second step in the biosynthesis of leucine. - Homoaconitase (EC 4.2.1.36) (homoaconitate hydratase), an enzyme that participates in the alpha-aminoadipate pathway of lysine biosynthesis and that converts cis-homoaconitate into homoisocitric acid. - *Escherichia coli* protein ybhJ. As a signature for proteins from the aconitase family, two conserved regions that contain the three cysteine ligands of the 4Fe-4S cluster were selected.

Consensus pattern: [LIVM]-x(2)-[GSACIVM]-x-[LIV]-[GTIV]-[STP]-C-x(0,1)-T-N-[GSTANI]-x(4)-[LIVMA] [C binds the iron-sulfur center]

Consensus pattern: G-x(2)-[LIVWPO]-x(3)-[GAC]-C-[GSTAM]-[LIMPTA]-C-[LIMV]-[GA] [The two C's bind the iron-sulfur center]

[1] Gruer M.J., Artymiuk P.J., Guest J.R. Trends Biochem. Sci. 22:3-6(1997).

19. (Acyl-CoA dh)

Acyl-CoA dehydrogenases signatures

Acyl-CoA dehydrogenases [1,2,3] are enzymes that catalyze the alpha, beta-dehydrogenation of acyl-CoA esters and transfer electrons to ETF, the electron transfer protein. Acyl-CoA dehydrogenases are FAD flavoproteins. This family currently includes: - Five eukaryotic isozymes that catalyze the first step of the beta-oxidation cycles for fatty acids with various chain lengths. These are short (SCAD) (EC 1.3.99.2), medium (MCAD) (EC 1.3.99.3), long (LCAD) (EC 1.3.99.13), very-long (VLCAD) and short/branched (SBCAD) chain acyl-CoA dehydrogenases. These enzymes are located in the mitochondrion. They are all

homotetrameric proteins of about 400 amino acid residues except VLCAD which is a dimer and which contains, in its mature form, about 600 residues. - Glutaryl-CoA dehydrogenase (EC 1.3.99.7) (GCDH), which is involved in the catabolism of lysine, hydroxylysine and tryptophan. - Isovaleryl-CoA dehydrogenase (EC 1.3.99.10) (IVD), involved in the catabolism of leucine. - Acyl-coA dehydrogenases acsA and mmgC from *Bacillus subtilis*. - Butyryl-CoA dehydrogenase (EC 1.3.99.2) from *Clostridium acetobutylicum*. - *Escherichia coli* protein caiA [4]. - *Escherichia coli* protein aidB. Two conserved regions were selected as signature patterns. The first is located in the center of these enzymes, the second in the C-terminal section.

10

Consensus pattern: [GAC]-[LIVM]-[ST]-E-x(2)-[GSAN]-G-[ST]-D-x(2)-[GSA]

Consensus pattern: [QDE]-x(2)-G-[GS]-x-G-[LIVMFY]-x(2)-[DEN]-x(4)-[KR]-x(3)-[DEN]

- 15 [1] Tanaka K., Ikeda, Matsubara Y., Hyman D.B. Enzyme 38:91-107(1987).
 [2] Matsubara Y., Indo Y., Naito E., Ozasa H., Glassberg R., Vockley J., Ikeda Y., Kraus J., Tanaka K. J. Biol. Chem. 264:16321-16331(1989).
 [3] Aoyama T., Ueno I., Kamijo T., Hashimoto T. J. Biol. Chem. 269:19088-19094(1994).
 [4] Eichler K., Bourgis F., Buchet A., Kleber H.-P., Mandrand-Berthelot M.-A. Mol. Microbiol. 13:775-786(1994).

20

20. (Acyl transf)

Acyl transferase domain

25

Number of members: 161

30

[1] Serre L., Verbree EC, Dauter Z, Stuitje AR, Derewenda ZS; Medline: 95286570 The *Escherichia coli* malonyl-CoA:acyl carrier protein transacylase at 1.5-Å resolution. Crystal structure of a fatty acid synthase component." J Biol Chem 1995;270:12961-12964.

21. Acylphosphatase signatures

Acylphosphatase (EC 3.6.1.7) [1,2] catalyzes the hydrolysis of various acylphosphate carboxyl-phosphate bonds such as carbamyl phosphate, succinylphosphate, 1,3-diphosphoglycerate, etc. The physiological role of this enzyme is not yet clear.

- Acylphosphatase is a small protein of around 100 amino-acid residues. There are two known isozymes. One seems to be specific to muscular tissues, the other, called 'organ-common type', is found in many different tissues. While acylphosphatase have been so far only characterized in vertebrates, there are a number of bacterial and archeobacterial hypothetical proteins that are highly similar to that enzyme and that probably possess the same activity. These proteins are: - *Escherichia coli* hypothetical protein yccX. - *Bacillus subtilis* hypothetical protein yfIL. - *Archaeoglobus fulgidus* hypothetical protein AF0818. Two conserved regions were selected as signature patterns. The first is located in the N-terminal section, while the second is found in the central part of the protein sequence.

Consensus pattern: [LIV]-x-G-x-V-Q-G-V-x-[FM]-R

Consensus pattern: G-[FYW]-[AVC]-[KROAM]-N-x(3)-G-x-V-x(5)-G

[1] Stefani M., Ramponi G. Life Chem. Rep. 12:271-301(1995).

[2] Stefani M., Taddei N., Ramponi G. Cell. Mol. Life Sci. 53:141-151(1997).

22. (Adap comp sub)

Clathrin adaptor complexes medium chain signatures.

- Clathrin coated vesicles (CCV) mediate intracellular membrane traffic such as receptor mediated endocytosis. In addition to clathrin, the CCV are composed of a number of other components including oligomeric complexes which are known as adaptor or clathrin assembly proteins (AP) complexes [1]. The adaptor complexes are believed to interact with the cytoplasmic tails of membrane proteins, leading to their selection and concentration. In mammals two type of adaptor complexes are known: AP-1 which is associated with the Golgi complex and AP-2 which is associated with the plasma membrane. Both AP-1 and AP-2 are heterotetramers that consist of two large chains - the adaptins - (gamma and beta' in AP-1; alpha and beta in AP-2); a medium chain (AP47 in AP-1; AP50 in AP-2) and a small chain (AP19 in AP-1; AP17 in AP-2). The medium chains of AP-1 and AP-2 are evolutionary

related proteins of about 50 Kd. Homologs of AP47 and AP50 have also been found in *Caenorhabditis elegans* (genes *unc-101* and *ap50*) [2] and yeast (gene *APM1* or *YAP54*) [3]. Some more divergent, but clearly evolutionary related proteins have also been found in yeast: *APM2* and *YBR288c*. Two conserved regions were selected as signature patterns, one
5 located in the N-terminal region, the other from the central section of these proteins.

Consensus pattern: [IVT]-[GSP]-W-R-x(2,3)-[GAD]-x(2)-[HY]-x(2)-N-x- [LIVMAFY](3)-
D-[LIVM]-[LIVMT]-E

10 Consensus pattern: [LIV]-x-F-I-P-P-x-G-x-[LIVMFY]-x-L-x(2)-Y

[1] Pearse B.M., Robinson M.S. *Annu. Rev. Cell Biol.* 6:151-171(1990).

[2] Lee J., Jongeward G.D., Sternberg P.W. *Genes Dev.* 8:60-73(1994).

[3] Nakayama Y., Goebel M., O'Brine G.B., Lemmon S., Pingchang C.E., Kirchhausen T.

15 *Eur. J. Biochem.* 202:569-574(1991).

23. (Adenylosucc synt)

Adenylosuccinate synthetase signatures

20

Adenylosuccinate synthetase (EC 6.3.4.4) [1] plays an important role in purinebiosynthesis, by catalyzing the GTP-dependent conversion of IMP and aspartic acid to AMP.

Adenylosuccinate synthetase has been characterized from various sources ranging from *Escherichia coli* (gene *purA*) to vertebrate tissues. Invertebrates, two isozymes are present -

25 one involved in purine biosynthesis and the other in the purine nucleotide cycle. Two conserved regions were selected as signature patterns. The first one is a perfectly conserved octapeptide located in the N-terminal section and which is involved in GTP-binding [2]. The second one includes a lysine residue known [2] to be essential for the enzyme's activity.

30 Consensus pattern: Q-W-G-D-E-G-K-G

Consensus pattern: G-I-[GR]-P-x-Y-x(2)-K-x(2)-R [K is the active site residue]

[1] Wiesmueller L., Wittbrodt J., Noegel A.A., Schleicher M. J. Biol. Chem. 266:2480-2485(1991).

[2] Silva M.M., Poland B.W., Hoffman C.R., Fromm H.J., Honzatko R.B. J. Mol. Biol. 254:431-446(1995).

5 [3] Bouyoub A., Barbier G., Forterre P., Labedan B. 2.3.CO;2-"J. Mol. Biol. 261:144-154(1996).

24. (AdoHcyase)

10 S-adenosyl-L-homocysteine hydrolase signatures

S-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) (AdoHcyase) is an enzyme of the activated methyl cycle, responsible for the reversible hydration of S-adenosyl-L-homocysteine into adenosine and homocysteine. AdoHcyase is an ubiquitous enzyme which
15 binds and requires NAD⁺ as a cofactor. AdoHcyase is a highly conserved protein [1] of about 430 to 470 amino acids. Two highly conserved regions were selected as signature patterns. The first pattern is located in the N-terminal section; the second is derived from aglycine-rich region in the central part of AdoHcyase; a region thought to be involved in NAD-binding.

20 Consensus pattern: [GSA]-[CS]-N-x-[FYLM]-S-[ST]-[QA]-[DEN]-x-[AV]-[AT]-[AD]-[AC]-[LIVMCG]

Consensus pattern: [GA]-[KS]-x(3)-[LIV]-x-G-[FY]-G-x-[VC]-G-[KRL]-G-x-[ASC]

25 [1] Sgama M.W., Aksamit R.R., Cantoni G.L., Bauer C.E. Proc. Natl. Acad. Sci. U.S.A. 89:6328-6332(1992).

25. AhpC/TSA family

30 This family contains proteins related to alkyl hydroperoxide reductase. Comment: (AhpC) and thiol specific antioxidant (TSA).

[1] Chae HZ, Robison K, Poole LB, Church G, Storz G, Rhee SG, Proc Natl Acad Sci U S A 1994;91:7017-7021

26. (Aldose epim)

- 5 Aldose 1-epimerase putative active site Aldose 1-epimerase (EC 5.1.3.3) (mutarotase) is the enzyme responsible for the anomeric interconversion of D-glucose and other aldoses between their alpha- and beta-forms. The sequence of mutarotase from two bacteria, *Acinetobacter calcoaceticus* and *Streptococcus thermophilus* is available [1]. It has also been shown that, on the basis of extensive sequence similarities, a mutarotase domain seem to be
10 present in the C-terminal half of the fungal GAL10 protein which encodes, in the N-terminal part, for UDP-glucose 4-epimerase. The best conserved region in the sequence of mutarotase is centered around a conserved histidine residue which may be involved in the catalytic mechanism.

- 15 Consensus pattern: [NS]-x-T-N-H-x-Y-[FW]-N-[LI]

[1] Poolman B., Royer T.J., Mainzer S.E., Schmidt B.F. J. Bacteriol. 172:4037-4047(1990).

20 27. (AlkA DNA repair)

Alkylbase DNA glycosidases alkA family signature

- Alkylbase DNA glycosidases [1] are DNA repair enzymes that hydrolyzes the deoxyribose N-glycosidic bond to excise various alkylated bases from a damaged DNA polymer. In
25 *Escherichia coli* there are two alkylbase DNA glycosidases: one (gene tag) which is constitutively expressed and which is specific for the removal of 3-methyladenine (EC 3.2.2.20), and one (gene alkA) which is induced during adaptation to alkylation and which can remove a variety of alkylation products (EC 3.2.2.21). Tag and alkA do not share any region of sequence similarity. In yeast there is an alkylbase DNA glycosidase (gene MAG1)
30 [2,3], which can remove 3-methyladenine or 7-methyladenine and which is structurally related to alkA. MAG and alkA are both proteins of about 300 amino acid residues. While the C- and N-terminal ends appear to be unrelated, there is a central region of about 130 residues which is well conserved. A portion of this region has been selected as a signature pattern.

Consensus pattern: G-I-G-x-W-[ST]-[AV]-x-[LIVMFY](2)-x-[LIVM]-x(8)-[MF]-x(2)-[ED]-D

- 5 [1] Lindahl T., Sedgwick B. *Annu. Rev. Biochem.* 57:133-157(1988).
 - [2] Bernal K.G., Bjoras M., Bjelland S., Seeberg E.C. *EMBO J.* 9:4563-4568(1990).
 - [3] Chen J., Derfler B., Samson L. *EMBO J.* 9:4569-4575(1990).
- 10 28. Ammonium transporters signature
- A number of proteins involved in the transport of ammonium ions across a membrane as well as some yet uncharacterized proteins have been shown [1,2] to be evolutionarily related. These proteins are: - Yeast ammonium transporters MEP1, MEP2 and MEP3. - *Arabidopsis thaliana* high affinity ammonium transporter (gene AMT1). - *Corynebacterium glutamicum* ammonium and methylammonium transport system. - *Escherichia coli* putative ammonium transporter amtB. - *Bacillus subtilis* nrgA. - *Mycobacterium tuberculosis* hypothetical protein MtCY338.09c. - *Synechocystis* strain PCC 6803 hypothetical proteins sl10108, sl10537 and sl11017. - *Methanococcus jannaschii* hypothetical proteins MJ0058 and MJ1343.
- 20 - *Caenorhabditis elegans* hypothetical proteins C05E11.4, F49E11.3 and M195.3. As expected by their transport function, these proteins are highly hydrophobic and seem to contain from 10 to 12 transmembrane domains. The best conserved region seems to be located in the fifth (or sixth) transmembrane region and is used as a signature pattern.
- 25 Consensus pattern: D-[FYWS]-A-G-[GSC]-x(2)-[IV]-x(3)-[SAG](2)-x(2)-[SAG]- [LIVMF]-x(3)-[LIVMFYWA](2)-x-[GK]-x-R
- [1] Ninnemann O., Janniaux J.-C., Frommer W.B. *EMBO J.* 13:3464-3471(1994).
 - [2] Siewe R.M., Weil B., Burkovski A., Eikmanns B.J., Eikmanns M., Kraemer R. *J. Biol. Chem.* 271:5398-5403(1996).
 - [3] Saier M.H. Jr. *Adv. Microbiol. Physiol.* 40:81-136(1998).
29. (Arch_histone)

CBF/NF-Y subunits signatures

Diverse DNA binding proteins are known to bind the CCAAT box, a common cis-acting element found in the promoter and enhancer regions of a large number of genes in eukaryotes. Amongst these proteins is one known as the CCAAT-binding factor (CBF) or NF-Y [1]. CBF is a heteromeric transcription factor that consists of two different components both needed for DNA-binding. The HAP protein complex of yeast binds to the upstream activation site of cytochrome C iso-1 gene (CYC1) as well as other genes involved in mitochondrial electron transport and activates their expression. It also recognizes the sequence CCAAT and is structurally and evolutionary related to CBF. The first subunit of CBF, known as CBF-A or NF-YB in vertebrates, HAP3 in budding yeast and as php3 in fission yeast, is a protein of 116 to 210 amino-acid residues which contains a highly conserved central domain of about 90 residues. This domain seems to be involved in DNA-binding; a signature pattern had been developed from its central part. The second subunit of CBF, known as CBF-B or NF-YA in vertebrates, HAP2 in budding yeast and php2 in fission yeast, is a protein of 265 to 350 amino-acid residues which contains a highly conserved region of about 60 residues. This region, called the 'essential core' [2], seems to consist of two subdomains: an N-terminal subunit-association domain and a C-terminal DNA recognition domain. A signature pattern has been developed from a section of the subunit-association domain.

Consensus pattern: C-V-S-E-x-I-S-F-[LIVM]-T-[SG]-E-A-[SC]-[DE]-[KRO]-C-

Consensus pattern: Y-V-N-A-K-Q-Y-x-R-I-L-K-R-R-x-A-R-A-K-L-E-

[1] Li X.-Y., Mantovani R., Hooft van Huijsduijnen R., Andre I., Benoist C., Mathis D. Nucleic Acids Res. 20:1087-1091(1992).

[2] Olesen J.T., Fikes J.D., Guarente L. Mol. Cell. Biol. 11:611-619(1991).

30. Argininosuccinate synthase signatures

Argininosuccinate synthase (EC 6.3.4.5) (AS) is a urea cycle enzyme that catalyzes the penultimate step in arginine biosynthesis: the ATP-dependent ligation of citrulline to

aspartate to form argininosuccinate, AMP and pyrophosphate [1,2]. In humans, a defect in the AS gene causes citrullinemia, a genetic disease characterized by severe vomiting spells and mental retardation. AS is a homotetrameric enzyme of chains of about 400 amino-acid residues. Arginine seems to be important for the enzyme's catalytic mechanism. The sequences of AS from various prokaryotes, archaeobacteria and eukaryotes show significant similarity. Two signature patterns have been selected for AS. The first is a highly conserved stretch of nine residues located in the N-terminal extremity of these enzymes, the second is derived from a conserved region which contains one of the conserved arginine residues.

10 Consensus pattern: [AS]-[FY]-S-G-G-[LV]-D-T-[ST]-

Consensus pattern: G-x-T-x-K-G-N-D-x(2)-R-F-

[1] van Vliet F., Crabeel M., Boyen A., Tricot C., Stalon V., Falmagne P., Nakamura Y.,

15 Baumberg S., Glansdorff N. *Gene* 95:99-104(1990).

[2] Morris C.J., Reeve J.N. *J. Bacteriol.* 170:3125-3130(1988).

31. Armadillo/beta-catenin-like repeats

20 Approx. 40 amino acid repeat. Tandem repeats form super-helix of helices that is proposed to mediate interaction of beta-catenin with its ligands. CAUTION: This family does not contain all known armadillo repeats.

[1] Huber AH, Nelson WJ, Weis WI, *Cell* 1997;90:871-882.

25 [2] Gumbiner BM, *Curr Opin Cell Biol* 1995;7:634-640.

[3] Cavallo R, Rubenstein D, Peifer M, *Curr Opin Genet Dev* 1997;7:459-466.

[4] Su LK, Vogelstein B, Kinzler KW, *Science* 1993;262:1734-1737.

[5] Masiarz FR, Munemitsu S, Polakis P *Science* 1993;262:1731-1734

[6] Peifer M, Wieschaus E, *Cell* 1990;63:1167-1176.

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32. (Asn Synthase)

Asparagine synthase

This family is always found associated with GATase_2. Members of this family catalyse the conversion of aspartate to asparagine.

5 33. Asparaginase_2

Asparaginase 12 members

34. (Aspartyl tRNA N)

10 Aminoacyl-transfer RNA synthetases class-II signatures

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA

- 15 synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. The synthetases specific for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is different to the Rossmann fold observed for the class I synthetases [7]. Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions are present [2,5,8]. Signature patterns have been derived from two of these regions.

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Consensus pattern: [FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE]

Consensus pattern: [GSTALVF]-[DENQHRKP]-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-[LIVMSTAG]-[LIVMFY]

30 [1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).

[2] Delarue M., Moras D. BioEssays 15:675-687(1993).

[3] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).

[4] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).

[5] Cusack S., Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991).

- [6] Cusack S. Biochimie 75:1077-1081(1993).
- [7] Cusack S., Berthet-Colominas C., Haertlein M., Nassar N., Leberman R. Nature 347:249-255(1990).
- [8] Leveque F., Plateau P., Dessen P., Blanquet S. Nucleic Acids Res. 18:305-312(1990).

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35. (ArfGap) Putative GTP-ase activating protein for Arf. Putative zinc fingers with GTPase activating proteins (GAPs) towards the small GTPase, Arf. The GAP of ARD1 stimulates GTPase hydrolysis for ARD1 but not ARFs. Number of members: 34

10

[1]Medline: 96324970. Identification and cloning of centaurin-alpha. A novel phosphatidylinositol 3,4,5-trisphosphate-binding protein from rat brain. Hammonds-Odie LP, Jackson TR, Profit AA, Blader IJ, Turck CW, Prestwich GD, Theibert AB; J Biol Chem 1996;271:18859-18868.

15

[2]Medline: 97296423. A target of phosphatidylinositol 3,4,5-trisphosphate with a zinc finger motif similar to that of the ADP-ribosylation -factor GTPase-activating protein and two pleckstrin homology domains. Tanaka K, Imajoh-Ohmi S, Sawada T, Shirai R, Hashimoto Y, Iwasaki S, Kaibuchi K, Kanaho Y, Shirai T, Terada Y, Kimura K, Nagata S, Fukui Y; Eur J Biochem 1997;245:512-519.

20

[3] 98112795. Molecular characterization of the GTPase-activating domain of ADP-ribosylation factor domain protein 1 (ARD1). Vitale N, Moss J, Vaughan M; J Biol Chem 1998;273:2553-2560.

25

36. Apolipoprotein. Apolipoprotein A1/A4/E family. This family includes: Swiss:P02647 Apolipoprotein A-I. Swiss:P06727 Apolipoprotein A-IV. Swiss:P02649 Apolipoprotein E. These proteins contain several 22 residue repeats which form a pair of alpha helices. Number of members: 42

30

[1]Medline: 91289138. Three-dimensional structure of the LDL receptor-binding domain of human apolipoprotein E. Wilson C, Wardell MR, Weisgraber KH, Mahley RW, Agard DA; Science 1991;252:1817-1822.

37. Amino acid permeases signature

Amino acid permeases are integral membrane proteins involved in the transport of amino acids into the cell. A number of such proteins have been found to be evolutionary related [1,2,3]. These proteins are: - Yeast general amino acid permeases (genes GAP1, AGP2 and AGP3). - Yeast basic amino acid permease (gene ALP1). - Yeast Leu/Val/Ile permease (gene BAP2). - Yeast arginine permease (gene CAN1). - Yeast dicarboxylic amino acid permease (gene DIP5). - Yeast asparagine/glutamine permease (gene AGP1). - Yeast glutamine permease (gene GNP1). - Yeast histidine permease (gene HIP1). - Yeast lysine permease (gene LYP1). - Yeast proline permease (gene PUT4). - Yeast valine and tyrosine permease (gene VAL1/TAT1). - Yeast tryptophan permease (gene TAT2/SCM2). - Yeast choline transport protein (gene HNM1/CTR1). - Yeast GABA permease (gene UGA4). - Yeast hypothetical protein YKL174c. - Fission yeast protein isp5. - Fission yeast hypothetical protein SpAC8A4.11 - Fission yeast hypothetical protein SpAC11D3.08c. - *Emericella nidulans* proline transport protein (gene *prnB*). - *Trichoderma harzianum* amino acid permease IND1. - *Salmonella typhimurium* L-asparagine permease (gene *ansP*). - *Escherichia coli* aromatic amino acid transport protein (gene *aroP*). - *Escherichia coli* D-serine/D-alanine/glycine transporter (gene *cycA*). - *Escherichia coli* GABA permease (gene *gabP*). - *Escherichia coli* lysine-specific permease (gene *lysP*). - *Escherichia coli* phenylalanine-specific permease (gene *pheP*). - *Salmonella typhimurium* proline-specific permease (gene *proY*). - *Escherichia coli* and *Klebsiella pneumoniae* hypothetical protein *yeeF*. - *Escherichia coli* and *Salmonella typhimurium* hypothetical protein *yifK*. - *Bacillus subtilis* permeases *rocC* and *rocE* which probably transports arginine or ornithine. These proteins seem to contain up to 12 transmembrane segments. As a signature for this family of proteins, the best conserved region which is located in the second transmembrane segment has been selected.

Consensus pattern: [STAGC]-G-[PAG]-x(2,3)-[LIVMFYWA](2)-x-[LIVMFYW]-x-[LIVMFSTAGC](2)-[STAGC]-x(3)-[LIVMFYWT]-x-[LIVMST]-x(3)-[LIVMCTA]-[GA]-E-x(5)-[PSAL]-

30

[1] Weber E., Chevalier M.R., Jund R. J. Mol. Evol. 27:341-350(1988).

[2] Vandenbol M., Jauniaux J.-C., Grenson M. Gene 83:153-159(1989).

[3] Reizer J., Finley K., Kakuda D., McLeod C.L., Reizer A., Saier M.H. Jr. Protein Sci. 2:20-30(1993).

38. aakinese (1) Glutamate 5-kinase signature

Glutamate 5-kinase (EC 2.7.2.11) (gamma-glutamyl kinase) (GK) is the enzyme that catalyzes the first step in the biosynthesis of proline from glutamate, the ATP-dependent phosphorylation of L-glutamate into L-glutamate 5-phosphate. In eubacteria (gene proB) and yeast [1] (gene PRO1), GK is a monofunctional protein, while in plants and mammals, it is a bifunctional enzyme (P5CS) [2] that consists of two domains: a N-terminal GK domain and a C-terminal gamma-glutamyl phosphate reductase domain (EC 1.2.1.41) (see <PDOC00940>). As a signature pattern, a highly conserved glycine-and alanine-rich region located in the central section of these enzymes has been selected. Yeast hypothetical protein YHR033w is highly similar to GK.

Consensus pattern: [GSTN]-x(2)-G-x-G-[GC]-[IM]-x-[STA]-K-[LIVM]-x-[SA]-[TCA]-x(2)-[GALV]-x(3)-G-

[1] Li W., Brandriss M.C. J. Bacteriol. 174:4148-4156(1992).

[2] Hu C.-A.A., Delauney A.J., Verma D.P.S. Proc. Natl. Acad. Sci. U.S.A. 89:9354-9358(1992).

aakinese (2) Aspartokinase signature

Aspartokinase (EC 2.7.2.4) (AK) [1] catalyzes the phosphorylation of aspartate. The product of this reaction can then be used in the biosynthesis of lysine or in the pathway leading to homoserine, which participates in the biosynthesis of threonine, isoleucine and methionine. In Escherichia coli, there are three different isozymes which differ in their sensitivity to repression and inhibition by Lys, Met and Thr. AK1 (gene thrA) and AK2 (gene metL) are bifunctional enzymes which both consist of an N- terminal AK domain and a C-terminal homoserine dehydrogenase domain. AK1 is involved in threonine biosynthesis and AK2, in that of methionine. The third isozyme, AK3 (gene lysC), is monofunctional and involved in lysine synthesis. In yeast, there is a single isozyme of AK (gene HOM3). As a signature pattern for AK, a conserved region located in the N-terminal extremity has been selected.

Consensus pattern: [LIVM]-x-K-[FY]-G-G-[ST]-[SC]-[LIVM]-

[1] Rafalski J.A., Falco S.C. J. Biol. Chem. 263:2146-2151(1988).

aakinase (3) Gamma-glutamyl phosphate reductase signature

Gamma-glutamyl phosphate reductase (EC 1.2.1.41) (GPR) is the enzyme that catalyzes the
 5 second step in the biosynthesis of proline from glutamate, the NADP-dependent reduction of
 L-glutamate 5-phosphate into L-glutamate 5-semialdehyde and phosphate. In eubacteria
 (gene proA) and yeast [1] (gene PRO2), GPR is a monofunctional protein, while in plants and
 mammals, it is a bifunctional enzyme (P5CS) [2] that consists of two domains: a N-terminal
 glutamate 5-kinase domain (EC 2.7.2.11) (see <PDOC00701>) and a C-terminal GPR
 10 domain. As a signature pattern, a conserved region that contains two histidine residues has
 been selected. This region is located in the last third of GPR.

Consensus pattern: V-x(5)-A-[LIV]-x-H-I-x(2)-[HY]-[GS]-[ST]-x-H-[ST]-[DE]-x- I-

15 [1] Pearson B.M., Hernando Y., Payne J., Wolf S.S., Kalogeropoulos A., Schweizer M.
 Yeast 12:1021-1031(1996).

[2] Hu C.-A.A., Delauney A.J., Verma D.P.S. Proc. Natl. Acad. Sci. U.S.A. 89:9354-
 9358(1992).

20 39. (abhydrolase) alpha/beta hydrolase fold. This catalytic domain is found in a very wide
 range of enzymes.

[1] Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolof F, Franken SM, Harel M, Remington
 25 SJ, Silman I, Schrag J, Sussman JL, Verschuere KHG, Goldman A, Protein Eng
 1992;5:197-211.

40. (Acid phosphat) Histidine acid phosphatases signatures

30 Acid phosphatases (EC 3.1.3.2) are a heterogeneous group of proteins that hydrolyze
 phosphate esters, optimally at low pH. It has been shown [1] that a number of acid
 phosphatases, from both prokaryotes and eukaryotes, share two regions of sequence
 similarity, each centered around a conserved histidine residue. These two histidines seem
 to be involved in the enzymes' catalytic mechanism [2,3]. The first histidine is located in the

N-terminal section and forms a phosphohistidine intermediate while the second is located in the C-terminal section and possibly acts as proton donor. Enzymes belonging to this family are called 'histidine acid phosphatases' and are listed below:

- *Escherichia coli* pH 2.5 acid phosphatase (gene *appA*).

5

- *Escherichia coli* glucose-1-phosphatase (EC 3.1.3.10) (gene *agp*).

- Yeast constitutive and repressible acid phosphatases (genes *PHO3* and *PHO5*).

- Fission yeast acid phosphatase (gene *pho1*).

- *Aspergillus* phytases A and B (EC 3.1.3.8) (gene *phyA* and *phyB*).

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- Mammalian lysosomal acid phosphatase.

- Mammalian prostatic acid phosphatase.

- *Caenorhabditis elegans* hypothetical proteins B0361.7, C05C10.1, C05C10.4 and F26C11.1.

15

Consensus pattern[LIVM]-x(2)-[LIVMA]-x(2)-[LIVM]-x-R-H-[GN]-x-R-x-[PAS] [H is the phosphohistidine residue]

Consensus pattern[LIVMF]-x-[LIVMFAG]-x(2)-[STAGI]-H-D-[STANQ]-x-[LIVM]-x(2)-[LIVMFY]-x(2)-[STA] [H is an active site residue] Sequences known to belong to this class

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detected by the patternALL, except for rat prostatic acid phosphatase which seems to have Tyr instead of the active site His

[1] van Etten R.L., Davidson R., Stevis P.E., MacArthur H., Moore D.L. J. Biol. Chem. 266:2313-2319(1991).

25

[2] Ostanin K., Harms E.H., Stevis P.E., Kuciel R., Zhou M.-M., van Etten R.L. J. Biol. Chem. 267:22830-22836(1992).

[3] Schneider G., Lindqvist Y., Vihko P. EMBO J. 12:2609-2615(1993).

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41. Aconitase family signatures

Aconitase (aconitate hydratase) (EC 4.2.1.3) [1] is the enzyme from the tricarboxylic acid cycle that catalyzes the reversible isomerization of citrate and isocitrate. Cis-aconitate is formed as an intermediary product during the course of the reaction. In eukaryotes two isozymes of aconitase are known to exist: one found in the mitochondrial matrix and the

other found in the cytoplasm. Aconitase, in its active form, contains a 4Fe-4S iron-sulfur cluster; three cysteine residues have been shown to be ligands of the 4Fe-4S cluster. It has been shown that the aconitase family also contains the following proteins: - Iron-responsive element binding protein (IRE-BP). IRE-BP is a cytosolic protein that binds to iron-responsive elements (IREs). IREs are stem-loop structures found in the 5'UTR of ferritin, and delta aminolevulinic acid synthase mRNAs, and in the 3'UTR of transferrin receptor mRNA. IRE-BP also express aconitase activity. - 3-isopropylmalate dehydratase (EC 4.2.1.33) (isopropylmalate isomerase), the enzyme that catalyzes the second step in the biosynthesis of leucine. - Homoaconitase (EC 4.2.1.36) (homoaconitate hydratase), an enzyme that participates in the alpha-aminoadipate pathway of lysine biosynthesis and that converts cis-homoaconitate into homoisocitric acid. - *Escherichia coli* protein ybhJ

Consensus pattern: [LIVM]-x(2)-[GSACIVM]-x-[LIV]-[GTIV]-[STP]-C-x(0,1)-T-N-[GSTANI]-x(4)-[LIVMA] [C binds the iron-sulfur center]

Consensus pattern: G-x(2)-[LIVWPO]-x(3)-[GAC]-C-[GSTAM]-[LIMPTA]-C-[LIMV]-[GA] [The two C's bind the iron-sulfur center]-

[1] Gruer M.J., Artymiuk P.J., Guest J.R. Trends Biochem. Sci. 22:3-6(1997).

42. Actins signatures

Actins [1 to 4] are highly conserved contractile proteins that are present in all eukaryotic cells. In vertebrates there are three groups of actin isoforms: alpha, beta and gamma. The alpha actins are found in muscle tissues and are a major constituent of the contractile apparatus. The beta and gamma actins co-exists in most cell types as components of the cytoskeleton and as mediators of internal cell motility. In plants [5] there are many isoforms which are probably involved in a variety of functions such as cytoplasmic streaming, cell shape determination, tip growth, graviperception, cell wall deposition, etc. Actin exists either in a monomeric form (G-actin) or in a polymerized form (F-actin). Each actin monomer can bind a molecule of ATP; when polymerization occurs, the ATP is hydrolyzed. Actin is a protein of from 374 to 379 amino acid residues. The structure of actin has been highly conserved in the course of evolution. Recently some divergent actin-like proteins have been identified in several species. These proteins are: - Centractin (actin-RPV) from mammals, fungi (yeast ACT5, *Neurospora crassa* ro-4) and *Pneumocystis carinii* (actin-II). Centractin

seems to be a component of a multi-subunit centrosomal complex involved in microtubule based vesicle motility. This subfamily is also known as ARP1. - ARP2 subfamily which includes chicken ACTL, yeast ACT2, Drosophila 14D, C.elegans actC. - ARP3 subfamily which includes actin 2 from mammals, Drosophila 66B, yeast ACT4 and fission yeast act2. -

5 ARP4 subfamily which includes yeast ACT3 and Drosophila 13E. Three signature patterns have been developed. The first two are specific to actins and span positions 54 to 64 and 357 to 365. The last signature picks up both actins and the actin-like proteins and corresponds to positions 106 to 118 in actins.

- 10 Consensus pattern: [FY]-[LIV]-G-[DE]-E-A-Q-x-[RKO](2)-G-
 Consensus pattern: W-[IV]-[STA]-[RK]-x-[DE]-Y-[DNE]-[DE]-
 Consensus pattern: [LM]-[LIVM]-T-E-[GAPO]-x-[LIVMFYWHO]-N-[PSTAQ]-x(2)-N-[KR]-
- 15 [1] Sheterline P., Clayton J., Sparrow J.C. (In) Actins, 3rd Edition, Academic Press Ltd, London, (1996).
 [2] Pollard T.D., Cooper J.A. Annu. Rev. Biochem. 55:987-1036(1986).
 [3] Pollard T.D. Curr. Opin. Cell Biol. 1:33-40(1990).
 [4] Rubenstein P.A. BioEssays 12:309-315(1990).
 20 [5] Meagher R.B., McLean B.G. Cell Motil. Cytoskeleton 16:164-166(1990).

43. Adenylate kinase signature

- Adenylate kinase (EC 2.7.4.3) (AK) [1] is a small monomeric enzyme that catalyzes the
- 25 reversible transfer of MgATP to AMP ($\text{MgATP} + \text{AMP} = \text{MgADP} + \text{ADP}$). In mammals there are three different isozymes: - AK1 (or myokinase), which is cytosolic. - AK2, which is located in the outer compartment of mitochondria. - AK3 (or GTP:AMP phosphotransferase), which is located in the mitochondrial matrix and which uses MgGTP instead of MgATP. The sequence of AK has also been obtained from different bacterial species and from plants and
- 30 fungi. Two other enzymes have been found to be evolutionary related to AK. These are: - Yeast uridylylate kinase (EC 2.7.4.-) (UK) (gene URA6) [2] which catalyzes the transfer of a phosphate group from ATP to UMP to form UDP and ADP. - Slime mold UMP-CMP kinase (EC 2.7.4.14) [3] which catalyzes the transfer of a phosphate group from ATP to either CMP or UMP to form CDP or UDP and ADP. Several regions of AK family enzymes are well

conserved, including the ATP-binding domains. The most conserved of all regions have been selected as a signature for this type of enzyme. This region includes an aspartic acid residue that is part of the catalytic cleft of the enzyme and that is involved in a salt bridge. It also includes an arginine residue whose modification leads to inactivation of the enzyme

5

Consensus pattern: [LIVMFYW](3)-D-G-[FYI]-P-R-x(3)-[NQ]-

[1] Schulz G.E. Cold Spring Harbor Symp. Quant. Biol. 52:429-439(1987).

[2] Liljelund P., Sanni A., Friesen J.D., Lacroute F. Biochem. Biophys. Res. Commun.

10 165:464-473(1989).

[3] Wiesmueller L., Noegel A.A., Barzu O., Gerisch G., Schleicher M. J. Biol. Chem.

265:6339-6345(1990).

[4] Kath T.H., Schmid R., Schaefer G. Arch. Biochem. Biophys. 307:405-410(1993).

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44. (adh_short) Short-chain dehydrogenases/reductases family signature. The short-chain dehydrogenases/reductases family (SDR) [1] is a very large family of enzymes, most of which are known to be NAD- or NADP-dependent oxidoreductases. As the first member of this family to be characterized was *Drosophila* alcohol dehydrogenase, this family used to be called [2,3,4] 'insect-type', or 'short-chain' alcohol dehydrogenases. Most member of this family are proteins of about 250 to 300 amino acid residues. The proteins currently known to belong to this family are listed below. - Alcohol dehydrogenase (EC 1.1.1.1) from insects such as *Drosophila*. - Acetoin dehydrogenase (EC 1.1.1.5) from *Klebsiella terrigena* (gene budC). - D-beta-hydroxybutyrate dehydrogenase (BDH) (EC 1.1.1.30) from mammals. - Acetoacetyl-CoA reductase (EC 1.1.1.36) from various bacterial species (gene phbB or phbB). - Glucose 1-dehydrogenase (EC 1.1.1.47) from *Bacillus*. - 3-beta-hydroxysteroid dehydrogenase (EC 1.1.1.51) from *Comomonas testosteroni*. - 20-beta-hydroxysteroid dehydrogenase (EC 1.1.1.53) from *Streptomyces hydrogenans*. - Ribitol dehydrogenase (EC 1.1.1.56) (RDH) from *Klebsiella aerogenes*. - Estradiol 17-beta-dehydrogenase (EC 1.1.1.62) from human. - Gluconate 5-dehydrogenase (EC 1.1.1.69) from *Gluconobacter oxydans* (gene gno). - 3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100) from *Escherichia coli* (gene fabG) and from plants. - Retinol dehydrogenase (EC 1.1.1.105) from mammals. - 2-deoxy-d-gluconate 3-dehydrogenase (EC 1.1.1.125) from *Escherichia coli* and *Erwinia chrysanthemi* (gene kduD). - Sorbitol-6-phosphate 2-dehydrogenase (EC 1.1.1.140) from *Escherichia coli*

30

- (gene *gutD*) and from *Klebsiella pneumoniae* (gene *sorD*). - 15-hydroxyprostaglandin dehydrogenase (NAD+) (EC 1.1.1.141) from human. - Corticosteroid 11-beta-dehydrogenase (EC 1.1.1.146) (11-DH) from mammals. - 7-alpha-hydroxysteroid dehydrogenase (EC 1.1.1.159) from *Escherichia coli* (gene *hdhA*), *Eubacterium* strain VPI 12708 (gene *baiA*) and
- 5 from *Clostridium sordellii*. - NADPH-dependent carbonyl reductase (EC 1.1.1.184) from mammals. - Tropinone reductase-I (EC 1.1.1.206) and -II (EC 1.1.1.236) from plants. - N-acylmannosamine 1-dehydrogenase (EC 1.1.1.233) from *Flavobacterium* strain 141-8. - D-arabinitol 2-dehydrogenase (ribulose forming) (EC 1.1.1.250) from fungi. -
- 10 Tetrahydroxynaphthalene reductase (EC 1.1.1.252) from *Magnaporthe grisea*. - Pteridine reductase 1 (EC 1.1.1.253) (gene *PTR1*) from *Leishmania*. - 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase (EC 1.1.-.-) from *Pseudomonas paucimobilis*. - Cis-1,2-dihydroxy-3,4-cyclohexadiene-1-carboxylate dehydrogenase (EC 1.3.1.-) from
- 15 *Acinetobacter calcoaceticus* (gene *benD*) and *Pseudomonas putida* (gene *xyll*). - Biphenyl-2,3-dihydro-2,3-diol dehydrogenase (EC 1.3.1.-) (gene *bphB*) from various *Pseudomonaceae*.
- Cis-toluene dihydrodiol dehydrogenase (EC 1.3.1.-) from *Pseudomonas putida* (gene *todD*). - Cis-benzene glycol dehydrogenase (EC 1.3.1.19) from *Pseudomonas putida* (gene *bnzE*). - 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) from *Escherichia coli* (gene
- 20 *entA*) and *Bacillus subtilis* (gene *dhbA*). - Dihydropteridine reductase (EC 1.6.99.7) (HDHPR) from mammals. - Lignin degradation enzyme *ligD* from *Pseudomonas paucimobilis*. - Agropine synthesis reductase from *Agrobacterium* plasmids (gene *mas1*). - Versicolorin reductase from *Aspergillus parasiticus* (gene *VER1*). - Putative keto-acyl reductases from *Streptomyces* polyketide biosynthesis operons. - A trifunctional hydratase-dehydrogenase-epimerase from the peroxisomal beta-oxidation system of *Candida tropicalis*. This protein contains two tandemly repeated 'short-chain dehydrogenase-type' domain in its
- 25 N-terminal extremity. - Nodulation protein *nodG* from species of *Azospirillum* and *Rhizobium* which is probably involved in the modification of the nodulation Nod factor fatty acyl chain. - Nitrogen fixation protein *fixR* from *Bradyrhizobium japonicum*. - *Bacillus subtilis* protein *dltE* which is involved in the biosynthesis of D- alanyl-lipoteichoic acid. - Human follicular variant translocation protein 1 (FVT1). - Mouse adipocyte protein p27. -
- 30 Mouse protein Ke 6. - Maize sex determination protein TASSELSEED 2. - *Sarcophaga peregrina* 25 Kd development specific protein. - *Drosophila* fat body protein P6. - A *Listeria monocytogenes* hypothetical protein encoded in the *internalins* gene region. - *Escherichia coli* hypothetical protein *yciK*. - *Escherichia coli* hypothetical protein *ydfG*. - *Escherichia coli* hypothetical protein *yjgI*. - *Escherichia coli* hypothetical protein *yjgU*. - *Escherichia coli*

hypothetical protein yohF. - *Bacillus subtilis* hypothetical protein yoxD. - *Bacillus subtilis* hypothetical protein ywFD. - *Bacillus subtilis* hypothetical protein ywIH. - Yeast hypothetical protein YIL124w. - Yeast hypothetical protein YIR035c. - Yeast hypothetical protein YIR036c. - Yeast hypothetical protein YKL055c. - Fission yeast hypothetical protein SpAC23D3.11. One of the best conserved regions which includes two perfectly conserved residues, a tyrosine and a lysine has been selected as a signature pattern for this family of proteins. The tyrosine residue participates in the catalytic mechanism.

Consensus pattern: [LIVSPADNK]-x(12)-Y-[PSTAGNCV]-[STAGNQCIWM]-[STAGC]-K-
 {PC}-[SAGFYR]-[LIVMSTAGD]-x(2)-[LIVMFYW]-x(3)-[LIVMFYWGAPTHQ]-
 [GSACQRHM] [Y is an active site residue] -

[1] Joernvall H., Persson B., Krook M., Atrian S., Gonzalez-Duarte R., Jeffery J., Ghosh D. *Biochemistry* 34:6003-6013(1995).

[2] Villarroya A., Juan E., Egestad B., Joernvall H. *Eur. J. Biochem.* 180:191-197(1989).

[3] Persson B., Krook M., Joernvall H. *Eur. J. Biochem.* 200:537-543(1991).

[4] Neidle E.L., Hartnett C., Ornston N.L., Bairoch A., Rekik M., Harayama S. *Eur. J. Biochem.* 204:113-120(1992).

45. (adh_short_C2) Short-chain dehydrogenases/reductases family signature

The short-chain dehydrogenases/reductases family (SDR) [1] is a very large family of enzymes, most of which are known to be NAD- or NADP-dependent oxidoreductases. As the first member of this family to be characterized was *Drosophila* alcohol dehydrogenase, this family used to be called [2,3,4]'insect-type', or 'short-chain' alcohol dehydrogenases. Most member of this family are proteins of about 250 to 300 amino acid residues. The proteins currently known to belong to this family are listed below. - Alcohol dehydrogenase (EC 1.1.1.1) from insects such as *Drosophila*. - Acetoin dehydrogenase (EC 1.1.1.5) from *Klebsiella terrigena* (gene budC). - D-beta-hydroxybutyrate dehydrogenase (BDH) (EC 1.1.1.30) from mammals. - Acetoacetyl-CoA reductase (EC 1.1.1.36) from various bacterial species (gene phbB or phaB). - Glucose 1-dehydrogenase (EC 1.1.1.47) from *Bacillus*. - 3-beta-hydroxysteroid dehydrogenase (EC 1.1.1.51) from *Comomonas testosteroni*. - 20-beta-hydroxysteroid dehydrogenase (EC 1.1.1.53) from *Streptomyces hydrogenans*. - Ribitol dehydrogenase (EC 1.1.1.56) (RDH) from *Klebsiella aerogenes*. - Estradiol 17-beta-

- dehydrogenase (EC 1.1.1.62) from human. - Gluconate 5-dehydrogenase (EC 1.1.1.69) from *Gluconobacter oxydans* (gene *gno*). - 3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100) from *Escherichia coli* (gene *fabG*) and from plants. - Retinol dehydrogenase (EC 1.1.1.105) from mammals. - 2-deoxy-d-gluconate 3-dehydrogenase (EC 1.1.1.125) from
- 5 *Escherichia coli* and *Erwinia chrysanthemi* (gene *kduD*). - Sorbitol-6-phosphate 2-dehydrogenase (EC 1.1.1.140) from *Escherichia coli* (gene *gutD*) and from *Klebsiella pneumoniae* (gene *sorD*). - 15-hydroxyprostaglandin dehydrogenase (NAD+) (EC 1.1.1.141) from human. - Corticosteroid 11-beta-dehydrogenase (EC 1.1.1.146) (11-DH) from mammals. - 7-alpha-hydroxysteroid dehydrogenase (EC 1.1.1.159) from *Escherichia coli*
- 10 (gene *hdhA*), *Eubacterium* strain VPI 12708 (gene *baiA*) and from *Clostridium sordellii*. - NADPH-dependent carbonyl reductase (EC 1.1.1.184) from mammals. - Tropinone reductase-I (EC 1.1.1.206) and -II (EC 1.1.1.236) from plants. - N-acylmannosamine 1-dehydrogenase (EC 1.1.1.233) from *Flavobacterium* strain I41-8. - D-arabinitol 2-dehydrogenase (ribulose forming) (EC 1.1.1.250) from fungi. - Tetrahydroxynaphthalene
- 15 reductase (EC 1.1.1.252) from *Magnaporthe grisea*. - Pteridine reductase I (EC 1.1.1.253) (gene *PTR1*) from *Leishmania*. - 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase (EC 1.1.-.) from *Pseudomonas paucimobilis*. - Cis-1,2-dihydroxy-3,4-cyclohexadiene-1-carboxylate dehydrogenase (EC 1.3.1.-) from *Acinetobacter calcoaceticus* (gene *benD*) and *Pseudomonas putida* (gene *xylL*). - Biphenyl-2,3-dihydro-2,3-diol dehydrogenase (EC 1.3.1.-)
- 20) (gene *bphB*) from various *Pseudomonaceae*. - Cis-toluene dihydrodiol dehydrogenase (EC 1.3.1.-) from *Pseudomonas putida* (gene *todD*). - Cis-benzene glycol dehydrogenase (EC 1.3.1.19) from *Pseudomonas putida* (gene *bnzE*). - 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) from *Escherichia coli* (gene *entA*) and *Bacillus subtilis* (gene *dhbA*). - Dihydropteridine reductase (EC 1.6.99.7) (HDHPR) from mammals. - Lignin
- 25 degradation enzyme *ligD* from *Pseudomonas paucimobilis*. - Agropine synthesis reductase from *Agrobacterium* plasmids (gene *mas1*). - Versicolorin reductase from *Aspergillus parasiticus* (gene *VER1*). - Putative keto-acyl reductases from *Streptomyces* polyketide biosynthesis operons. - A trifunctional hydratase-dehydrogenase-epimerase from the peroxisomal beta-oxidation system of *Candida tropicalis*. This protein contains two tandemly
- 30 repeated 'short-chain dehydrogenase-type' domain in its N-terminal extremity. - Nodulation protein *nodG* from species of *Azospirillum* and *Rhizobium* which is probably involved in the modification of the nodulation Nod factor fatty acyl chain. - Nitrogen fixation protein *fixR* from *Bradyrhizobium japonicum*. - *Bacillus subtilis* protein *dltE* which is involved in the biosynthesis of D- alanyl-lipoteichoic acid. - Human follicular variant translocation protein 1

(FVT1). - Mouse adipocyte protein p27. - Mouse protein Ke 6. - Maize sex determination protein TASSELSEED 2. - Sarcophaga peregrina 25 Kd development specific protein. - Drosophila fat body protein P6. - A *Listeria monocytogenes* hypothetical protein encoded in the internalins gene region. - *Escherichia coli* hypothetical protein yciK. - *Escherichia coli* hypothetical protein ydfG. - *Escherichia coli* hypothetical protein yjgI. - *Escherichia coli* hypothetical protein yjgU. - *Escherichia coli* hypothetical protein yohF. - *Bacillus subtilis* hypothetical protein yoxD. - *Bacillus subtilis* hypothetical protein ywID. - *Bacillus subtilis* hypothetical protein ywfH. - Yeast hypothetical protein YIL124w. - Yeast hypothetical protein YIR035c. - Yeast hypothetical protein YIR036c. - Yeast hypothetical protein YKL055c. - Fission yeast hypothetical protein SpAC23D3.11. One of the best conserved regions which includes two perfectly conserved residues, a tyrosine and a lysine has been used as a signature pattern for this family of proteins. The tyrosine residue participates in the catalytic mechanism.

Consensus pattern: [LIVSPADNK]-x(12)-Y-[PSTAGNCV]-[STAGNQCI VM]-[STAGC]-K-{PC}-[SAGFYR]-[LIVMSTAGD]-x(2)-[LIVMFYW]-x(3)-[LIVMFYWGAPTHQ]-[GSACQRHM] [Y is an active site residue]

[1] Joernvall H., Persson B., Krook M., Atrian S., Gonzalez-Duarte R., Jeffery J., Ghosh D.

Biochemistry 34:6003-6013(1995).

[2] Villarroya A., Juan E., Egestad B., Joernvall H. Eur. J. Biochem. 180:191-197(1989).

[3] Persson B., Krook M., Joernvall H. Eur. J. Biochem. 200:537-543(1991).

[4] Neidle E.L., Hartnett C., Ornston N.L., Bairoch A., Reikik M., Harayama S. Eur. J. Biochem. 204:113-120(1992).

46. (adh_zinc) Zinc-containing alcohol dehydrogenases signatures

Alcohol dehydrogenase (EC 1.1.1.1) (ADH) catalyzes the reversible oxidation of ethanol to acetaldehyde with the concomitant reduction of NAD [1]. Currently three, structurally and

catalytically, different types of alcohol dehydrogenases are known: - Zinc-containing 'long-chain' alcohol dehydrogenases. - Insect-type, or 'short-chain' alcohol dehydrogenases. - Iron-containing alcohol dehydrogenases. Zinc-containing ADH's [2,3] are dimeric or tetrameric enzymes that bind two atoms of zinc per subunit. One of the zinc atom is essential for catalytic activity while the other is not. Both zinc atoms are coordinated by either cysteine or

- histidine residues; the catalytic zinc is coordinated by two cysteines and one histidine. Zinc-containing ADH's are found in bacteria, mammals, plants, and in fungi. In most species there are more than one isozyme (for example, human have at least six isozymes, yeast have three, etc.). A number of other zinc-dependent dehydrogenases are closely related to zinc ADH [4],
- 5 these are: - Xylitol dehydrogenase (EC 1.1.1.9) (D-xylulose reductase). - Sorbitol dehydrogenase (EC 1.1.1.14). - Aryl-alcohol dehydrogenase (EC 1.1.1.90) (benzyl alcohol dehydrogenase). - Threonine 3-dehydrogenase (EC 1.1.1.103). - Cinnamyl-alcohol dehydrogenase (EC 1.1.1.195) (CAD) [5]. CAD is a plant enzyme involved in the biosynthesis of lignin. - Galactitol-1-phosphate dehydrogenase (EC 1.1.1.251). -
- 10 *Pseudomonas putida* 5-exo-alcohol dehydrogenase (EC 1.1.1.-) [6]. - *Escherichia coli* starvation sensing protein *rspB*. - *Escherichia coli* hypothetical protein *yjgB*. - *Escherichia coli* hypothetical protein *yjgV*. - *Escherichia coli* hypothetical protein *yjjN*. - Yeast hypothetical protein YAL060w (FUN49). - Yeast hypothetical protein YAL061w (FUN50). - Yeast hypothetical protein YCR105w. The pattern that has been developed to detect this class
- 15 of enzymes is based on a conserved region that includes a histidine residue which is the second ligand of the catalytic zinc atom. This family also includes NADP-dependent quinone oxidoreductase (EC 1.6.5.5), an enzyme found in bacteria (gene *qor*), in yeast and in mammals where, in some species such as rodents, it has been recruited as an eye lens protein and is known as zeta-crystallin [7]. The sequence of quinone oxidoreductase is distantly
- 20 related to that other zinc-containing alcohol dehydrogenases and it lacks the zinc-ligand residues. The torpedo fish and mammalian synaptic vesicle membrane protein *vat-1* is related to *qor*. A specific pattern has been developed for this subfamily.

Consensus pattern: G-H-E-x(2)-G-x(5)-[GA]-x(2)-[IVSAC] [H is a zinc ligand]

- 25 Consensus pattern: [GSD]-[DEQH]-x(2)-L-x(3)-[SA](2)-G-G-x-G-x(4)-Q-x(2)-[KR]-

[1] Branden C.-I., Joernvall H., Eklund H., Furugren B. (In) The Enzymes (3rd edition) 11:104-190(1975).

[2] Joernvall H., Persson B., Jeffery J. Eur. J. Biochem. 167:195-201(1987).

- 30 [3] Sun H.-W., Plapp B.V. J. Mol. Evol. 34:522-535(1992).

[4] Persson B., Hallborn J., Walfridsson M., Hahn-Haegerdal B., Keraenen S., Penttilae M., Joernvall H. FEBS Lett. 324:9-14(1993).

[5] Knight M.E., Halpin C., Schuch W. Plant Mol. Biol. 19:793-801(1992).

[6] Koga H., Aramaki H., Yamaguchi E., Takeuchi K., Horiuchi T., Gunsalus I.C. J. Bacteriol. 166:1089-1095(1986).

[7] Joernvall H., Persson B., Du Bois G., Lavers G.C., Chen J.H., Gonzalez P., Rao P.V., Zigler J.S. Jr. FEBS Lett. 322:240-244(1993).

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47. (aldehyd) Aldehyde dehydrogenases active sites

Aldehyde dehydrogenases (EC 1.2.1.3 and EC 1.2.1.5) are enzymes which oxidize a wide variety of aliphatic and aromatic aldehydes. In mammals at least four different forms of the enzyme are known [1]: class-1 (or Ald C) a tetrameric cytosolic enzyme, class-2 (or Ald M) a tetrameric mitochondrial enzyme, class-3 (or Ald D) a dimeric cytosolic enzyme, and class IV a microsomal enzyme. Aldehyde dehydrogenases have also been sequenced from fungal and bacterial species. A number of enzymes are known to be evolutionary related to aldehyde dehydrogenases; these enzymes are listed below. - Plants and bacterial betaine-aldehyde dehydrogenase (EC 1.2.1.8) [2], an enzyme that catalyzes the last step in the biosynthesis of betaine. - Plants and bacterial NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.9). - Escherichia coli succinate-semialdehyde dehydrogenase (NADP+) (EC 1.2.1.16) (gene gabD) [3], which reduces succinate semialdehyde into succinate. - Escherichia coli lactaldehyde dehydrogenase (EC 1.2.1.22) (gene ald) [4]. - Mammalian succinate semialdehyde dehydrogenase (NAD+) (EC 1.2.1.24). - Escherichia coli phenylacetaldehyde dehydrogenase (EC 1.2.1.39). - Escherichia coli 5-carboxymethyl-2-hydroxyumuconate semialdehyde dehydrogenase (gene hpcC). - Pseudomonas putida 2-hydroxyumuconic semialdehyde dehydrogenase [5] (genes dmpC and xylG), an enzyme in the meta-cleavage pathway for the degradation of phenols, cresols and catechol. - Bacterial and mammalian methylmalonate-semialdehyde dehydrogenase (MMSDH) (EC 1.2.1.27) [6], an enzyme involved in the distal pathway of valine catabolism. - Yeast delta-1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12) [7] (gene PUT2), which converts proline to glutamate. - Bacterial multifunctional putA protein, which contains a delta-1-pyrroline-5-carboxylate dehydrogenase domain. - 26G, a garden pea protein of unknown function which is induced by dehydration of shoots [8]. - Mammalian formyltetrahydrofolate dehydrogenase (EC 1.5.1.6) [9]. This is a cytosolic enzyme responsible for the NADP-dependent decarboxylative reduction of 10-formyltetrahydrofolate into tetrahydrofolate. It is an protein of about 900 amino acids which consist of three domains; the C- terminal domain (480 residues) is structurally and functionally related to aldehyde dehydrogenases. - Yeast

hypothetical protein YBR006w. - Yeast hypothetical protein YER073w. - Yeast hypothetical protein YHR039c. - *Caenorhabditis elegans* hypothetical protein F01F1.6.A glutamic acid and a cysteine residue have been implicated in the catalytic activity of mammalian aldehyde dehydrogenase. These residues are conserved in all the enzymes of this family. Two patterns

- 5 have been derived for this family, one for each of the active site residues.

Consensus pattern: [LIVMFGA]-E-[LIMSTAC]-[GS]-G-[KNLM]-[SADN]-[TAPFV] [E is the active site residue]-

Consensus pattern: [FYLVA]-x(3)-G-[QE]-x-C-[LIVMGSTANC]-[AGCN]-x-

- 10 [GSTADNEKR] [C is the active site residue]

[1] Hempel J., Harper K., Lindahl R. *Biochemistry* 28:1160-1167(1989).

[2] Weretilnyk E.A., Hanson A.D. *Proc. Natl. Acad. Sci. U.S.A.* 87:2745-2749(1990).

[3] Niegemann E., Schulz A., Bartsch K. *Arch. Microbiol.* 160:454-460(1993).

- 15 [4] Hidalgo E., Chen Y.-M., Lin E.C.C., Aguilar J. J. *Bacteriol.* 173:6118-6123(1991).

[5] Nordlund I., Shingler V. *Biochim. Biophys. Acta* 1049:227-230(1990).

[6] Steele M.I., Lorenz D., Hatter K., Park A., Sokatch J.R. *J. Biol. Chem.* 267:13585-13592(1992).

[7] Krzywicki K.A., Brandriss M.C. *Mol. Cell. Biol.* 4:2837-2842(1984).

- 20 [8] Guerrero F.D., Jones J.T., Mullet J.E. *Plant Mol. Biol.* 15:11-26(1990).

[9] Cook R.J., Lloyd R.S., Wagner C. J. *Biol. Chem.* 266:4965-4973(1991).

48. Aldo/keto reductase family signatures

- 25 The aldo-keto reductase family [1,2] groups together a number of structurally and functionally related NADPH-dependent oxidoreductases as well as some other proteins. The proteins known to belong to this family are: - Aldehyde reductase (EC 1.1.1.2). - Aldose reductase (EC 1.1.1.21). - 3-alpha-hydroxysteroid dehydrogenase (EC 1.1.1.50), which terminates androgen action by converting 5-alpha-dihydrotestosterone to 3-alpha-androstanediol. - Prostaglandin F synthase (EC 1.1.1.188) which catalyzes the reduction of prostaglandins H2 and D2 to F2-alpha. - D-sorbitol-6-phosphate dehydrogenase (EC 1.1.1.200) from apple. - Morphine 6-dehydrogenase (EC 1.1.1.218) from *Pseudomonas putida* plasmid pMDH7.2 (gene morA). - Chlordecone reductase (EC 1.1.1.225) which reduces the pesticide chlordecone (kepone) to the corresponding alcohol. - 2,5-diketo-D-
- 30

gluconic acid reductase (EC 1.1.1.-) which catalyzes the reduction of 2,5-diketogluconic acid to 2-keto-L-gulononic acid, a key intermediate in the production of ascorbic acid. - NAD(P)H-dependent xylose reductase (EC 1.1.1.-) from the yeast *Pichia stipitis*. This enzyme reduces xylose into xylitol. - Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase (EC 1.3.1.20). - 3-oxo-5-beta-steroid 4-dehydrogenase (EC 1.3.99.6) which catalyzes the reduction of Δ^4 -3-oxosteroids. - A soybean reductase, which co-acts with chalcone synthase in the formation of 4,2',4'-trihydroxychalcone. - Frog eye lens rho crystallin. - Yeast GCY protein, whose function is not known. - *Leishmania major* P110/11E protein. P110/11E is a developmentally regulated protein whose abundance is markedly elevated in promastigotes compared with amastigotes. Its exact function is not yet known. - *Escherichia coli* hypothetical protein yafB. - *Escherichia coli* hypothetical protein yghE. - Yeast hypothetical protein YBR149w. - Yeast hypothetical protein YHR104w. - Yeast hypothetical protein YJR096w. These proteins have all about 300 amino acid residues. Three consensus patterns have been developed that are specific to this family of proteins. The first one is located in the N-terminal section of these proteins. The second pattern is located in the central section. The third pattern, located in the C-terminal, is centered on a lysine residue whose chemical modification, in aldose and aldehydereductases, affect the catalytic efficiency.

Consensus pattern: G-[FY]-R-[HSAL]-[LIVMF]-D-[STAGC]-[AS]-x(5)-E-x(2)-[LIVM]-G -

Consensus pattern: [LIVMFY]-x(9)-[KREQ]-x-[LIVM]-G-[LIVM]-[SC]-N-[FY]-

Consensus pattern: [LIVM]-[PAIV]-[KR]-[ST]-x(4)-R-x(2)-[GSTAEQK]-[NSL]-x(2)-[LIVMFA] [K is a putative active site residue]-

[1] Bohren K.M., Bullock B., Wermuth B., Gabbay K.H. J. Biol. Chem. 264:9547-

9551(1989).

[2] Bruce N.C., Willey D.L., Coulson A.F.W., Jeffery J. Biochem. J. 299:805-811(1994).

49. Alpha amylase. This family is classified as family 13 of the glycosyl hydrolases. The structure is an 8 stranded alpha/beta barrel, interrupted by a ~70 a.a. calcium-binding domain protruding between beta strand 3 and alpha helix 3, and a carboxyl-terminal Greek key beta-barrel domain.

[1] Larson SB, Greenwood A, Cascio D, Day J, McPherson A, J Mol Biol 1994;235:1560-1584.

5 50. Aminotransferases class-I pyridoxal-phosphate attachment site

Aminotransferases share certain mechanistic features with other pyridoxal- phosphate dependent enzymes, such as the covalent binding of the pyridoxal- phosphate group to a lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1,2] into subfamilies. One of these, called class-I, currently consists of the following
 10 enzymes: - Aspartate aminotransferase (AAT) (EC 2.6.1.1). AAT catalyzes the reversible transfer of the amino group from L-aspartate to 2-oxoglutarate to form oxaloacetate and L- glutamate. In eukaryotes, there are two AAT isozymes: one is located in the mitochondrial matrix, the second is cytoplasmic. In prokaryotes, only one form of AAT is found (gene aspC). - Tyrosine aminotransferase (EC 2.6.1.5) which catalyzes the first step in tyrosine
 15 catabolism by reversibly transferring its amino group to 2- oxoglutarate to form 4- hydroxyphenylpyruvate and L-glutamate. - Aromatic aminotransferase (EC 2.6.1.57) involved in the synthesis of Phe, Tyr, Asp and Leu (gene tyrB). - 1-aminocyclopropane-1- carboxylate synthase (EC 4.4.1.14) (ACC synthase) from plants. ACC synthase catalyzes the first step in ethylene biosynthesis. - Pseudomonas denitrificans cobC, which is involved in
 20 cobalamin biosynthesis. - Yeast hypothetical protein YJL060w. The sequence around the pyridoxal-phosphate attachment site of this class of enzyme is sufficiently conserved to allow the creation of a specific pattern.

Consensus pattern: [GS]-[LIVMFYTAC]-[GSTA]-K-x(2)-[GSALVN]-[LIVMFA]-x-
 25 [GNAR]- x-R-[LIVMA]-[GA] [K is the pyridoxal-P attachment site]

[1] Bairoch A. Unpublished observations (1992).

[2] Sung M.H., Tanizawa K., Tanaka H., Kuramitsu S., Kagamiyama H., Hirotsu K., Okamoto A., Higuchi T., Soda K. J. Biol. Chem. 266:2567-2572(1991).

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51. Aminotransferases class-II pyridoxal-phosphate attachment site

Aminotransferases share certain mechanistic features with other pyridoxal- phosphate dependent enzymes, such as the covalent binding of the pyridoxal- phosphate group to a

lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1] into subfamilies. One of these, called class-II, currently consists of the following enzymes: - Glycine acetyltransferase (EC 2.3.1.29), which catalyzes the addition of acetyl-CoA to glycine to form 2-amino-3-oxobutanoate (gene kbl). - 5-aminolevulinic acid synthase (EC 2.3.1.37) (delta-ALA synthase), which catalyzes the first step in heme biosynthesis via the Shemin (or C4) pathway, i.e. the addition of succinyl-CoA to glycine to form 5-aminolevulinate. - 8-amino-7-oxononanoate synthase (EC 2.3.1.47) (7-KAP synthetase), a bacterial enzyme (gene bioF) which catalyzes an intermediate step in the biosynthesis of biotin: the addition of 6-carboxy-hexanoyl-CoA to alanine to form 8-amino-7-oxononanoate. - Histidinol-phosphate aminotransferase (EC 2.6.1.9), which catalyzes the eighth step in histidine biosynthetic pathway: the transfer of an amino group from 3-(imidazol-4-yl)-2-oxopropyl phosphate to glutamic acid to form histidinol phosphate and 2-oxoglutarate. - Serine palmitoyltransferase (EC 2.3.1.50) from yeast (genes LCB1 and LCB2), which catalyzes the condensation of palmitoyl-CoA and serine to form 3- ketosphinganine. The sequence around the pyridoxal-phosphate attachment site of this class of enzyme is sufficiently conserved to allow the creation of a specific pattern

Consensus pattern: T-[LIVMFYW]-[STAG]-K-[SAG]-[LIVMFYWR]-[SAG]-x(2)-[SAG]
[K is the pyridoxal-P attachment site]-

[1] Bairoch A. Unpublished observations (1991).

52. Aminotransferases class-III pyridoxal-phosphate attachment site

Aminotransferases share certain mechanistic features with other pyridoxal- phosphate dependent enzymes, such as the covalent binding of the pyridoxal- phosphate group to a lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1,2] into subfamilies. One of these, called class-III, currently consists of the following enzymes: - Acetylornithine aminotransferase (EC 2.6.1.11) which catalyzes the transfer of an amino group from acetylornithine to alpha-ketoglutarate, yielding N-acetyl- glutamic-5-semi-aldehyde and glutamic acid. - Ornithine aminotransferase (EC 2.6.1.13), which catalyzes the transfer of an amino group from ornithine to alpha-ketoglutarate, yielding glutamic-5- semi-aldehyde and glutamic acid. - Omega-amino acid-pyruvate aminotransferase (EC 2.6.1.18), which catalyzes transamination between a variety of omega-amino acids, mono- and

diamines, and pyruvate. It plays a pivotal role in omega amino acids metabolism. - 4-aminobutyrate aminotransferase (EC 2.6.1.19) (GABA transaminase), which catalyzes the transfer of an amino group from GABA to alpha-ketoglutarate, yielding succinate semialdehyde and glutamic acid. - DAPA aminotransferase (EC 2.6.1.62), a bacterial enzyme (gene bioA) which catalyzes an intermediate step in the biosynthesis of biotin, the transamination of 7-keto-8-aminopelargonic acid (7-KAP) to form 7,8- diaminopelargonic acid (DAPA). - 2,2-dialkylglycine decarboxylase (EC 4.1.1.64), a *Pseudomonas cepacia* enzyme (gene dgdA) that catalyzes the decarboxylating amino transfer of 2,2-dialkylglycine and pyruvate to dialkyl ketone, alanine and carbon dioxide. - Glutamate-1-semialdehyde aminotransferase (EC 5.4.3.8) (GSA). GSA is the enzyme involved in the second step of porphyrin biosynthesis, via the C5 pathway. It transfers the amino group on carbon 2 of glutamate-1- semialdehyde to the neighbouring carbon, to give delta-aminolevulinic acid. - *Bacillus subtilis* aminotransferase yhxA. - *Bacillus subtilis* aminotransferase yodT. - *Haemophilus influenzae* aminotransferase HI0949. - *Caenorhabditis elegans* aminotransferase T01B11.2. The sequence around the pyridoxal-phosphate attachment site of this class of enzyme is sufficiently conserved to allow the creation of a specific pattern.

Consensus pattern: [LIVMFYWC](2)-x-D-E-[IVA]-x(2)-G-[LIVMFAGC]-x(0,1)-[RSACLI]-x-[GSAD]-x(12,16)-D-[LIVMFC]-[LIVMFYSTA]-x(2)- [GSA]-K-x(3)-[GSTADNV]-[GSAC] [K is the pyridoxal-P attachment site]-

[1] Bairoch A. Unpublished observations (1992).[2] Yonaha K., Nishie M., Aibara S. J. Biol. Chem. 267:12506-12510(1992).

53. Ank repeat. There's no clear separation between noise and signal on the HMM search. Ankyrin repeats generally consist of a beta, alpha, alpha, beta order of secondary structures. The repeats associate to form a higher order structure.

[1] A, Holak TA, FEBS Lett 1997;401:127-132.

[2] Lux SE, John KM, Bennett V, Nature 1990;345:736-739.

54. Aminotransferases class-IV signature

Aminotransferases share certain mechanistic features with other pyridoxal-phosphate dependent enzymes, such as the covalent binding of the pyridoxal-phosphate group to a lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1,2] into subfamilies. One of these, called class-IV, currently consists of the following

5 enzymes:

- Branched-chain amino-acid aminotransferase (EC 2.6.1.42) (transaminase B), a bacterial (gene *ilvE*) and eukaryotic enzyme which catalyzes the reversible transfer of an amino group from 4-methyl-2-oxopentanoate to glutamate, to form leucine and 2-oxoglutarate.
- 10 - D-alanine aminotransferase (EC 2.6.1.21). A bacterial enzyme which catalyzes the transfer of the amino group from D-alanine (and other D-amino acids) to 2-oxoglutarate, to form pyruvate and D-aspartate.
- 4-amino-4-deoxychorismate (ADC) lyase (gene *pabC*). A bacterial enzyme that converts ADC into 4-aminobenzoate (PABA) and pyruvate.

15 The above enzymes are proteins of about 270 to 415 amino-acid residues that share a few regions of sequence similarity. Surprisingly, the best-conserved region does not include the lysine residue to which the pyridoxal-phosphate group is known to be attached, in *ilvE*. The region that has been selected as a signature pattern is located some 40 residues at the C-terminus side of the PIP-lysine

20 Consensus pattern: E-x-[STAGCI]-x(2)-N-[LIVMFAC]-[FY]-x(6,12)-[LIVMF]-x-T- x(6,8)-[LIVM]-x-[GS]-[LIVM]-x-[KR]-

[1] Green J.M., Merkel W.K., Nichols B.P. J. Bacteriol. 174:5317-5323(1992).

25 [2] Bairoch A. Unpublished observations (1992).

55. Aminotransferases class-V pyridoxal-phosphate attachment site

Aminotransferases share certain mechanistic features with other pyridoxal- phosphate dependent enzymes, such as the covalent binding of the pyridoxal- phosphate group to a lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1,2] into subfamilies. One of these, called class-V, currently consists of the following enzymes: - Phosphoserine aminotransferase (EC 2.6.1.52), an enzyme which catalyzes the reversible interconversion of phosphoserine and 2-oxoglutarate to 3-phosphonooxypyruvate and glutamate. It is required both in the major phosphorylated pathway of serine biosynthesis

and in pyridoxine biosynthesis. The bacterial enzyme (gene serC) is highly similar to a rabbit endometrial progesterone-induced protein (EPIP), which is probably a phosphoserine aminotransferase [3]. - Serine--glyoxylate aminotransferase (EC 2.6.1.45) (SGAT) (gene sgaA) from *Methylobacterium extorquens*. - Serine--pyruvate aminotransferase (EC 2.6.1.51). This enzyme also acts as an alanine--glyoxylate aminotransferase (EC 2.6.1.44). In vertebrates, it is located in the peroxisomes and/or mitochondria. - Isopenicillin N epimerase (gene cefD). This enzyme is involved in the biosynthesis of cephalosporin antibiotics and catalyzes the reversible isomerization of isopenicillin N and penicillin N. - NifS, a protein of the nitrogen fixation operon of some bacteria and cyanobacteria. The exact function of nifS is not yet known. A highly similar protein has been found in fungi (gene NFS1 or SPL1). - The small subunit of cyanobacterial soluble hydrogenase (EC 1.12.-.-). - Hypothetical protein ycbU from *Bacillus subtilis*. - Hypothetical protein YFL030w from yeast. The sequence around the pyridoxal-phosphate attachment site of this class of enzyme is sufficiently conserved to allow the creation of a specific pattern.

Consensus pattern: [LIVFYCHT]-[DGH]-[LIVMFYAC]-[LIVMFYA]-x(2)-[GSTAC]-[GSTA]-[HQR]-K-x(4,6)-G-x-[GSAT]-x-[LIVMFYSAC] [K is the pyridoxal-P attachment site]-

[1] Ouzounis C., Sander C. FEBS Lett. 322:159-164(1993).

[2] Bairoch A. Unpublished observations (1992).

[3] van der Zel A., Lam H.-M., Winkler M.E. Nucleic Acids Res. 17:8379-8379(1989).

56. Annexins repeated domain signature

Annexins [1 to 6] are a group of calcium-binding proteins that associate reversibly with membranes. They bind to phospholipid bilayers in the presence of micromolar free calcium concentration. The binding is specific for calcium and for acidic phospholipids. Annexins have been claimed to be involved in cytoskeletal interactions, phospholipase inhibition, intracellular signalling, anticoagulation, and membrane fusion. Each of these proteins consist of an N-terminal domain of variable length followed by four or eight copies of a conserved segment of sixty one residues. The repeat (sometimes known as an 'endonexin fold') consists of five alpha-helices that are wound into a right-handed superhelix [7]. The proteins known to belong to the annexin family are listed below: - Annexin I (Lipocortin 1) (Calpactin 2) (p35)

(Chromobindin 9). - Annexin II (Lipocortin 2) (Calpactin 1) (Protein I) (p36) (Chromobindin 8). - Annexin III (Lipocortin 3) (PAP-III). - Annexin IV (Lipocortin 4) (Endonexin I) (Protein II) (Chromobindin 4). - Annexin V (Lipocortin 5) (Endonexin 2) (VAC-alpha) (Anchorin CII) (PAP-I). - Annexin VI (Lipocortin 6) (Protein III) (Chromobindin 20) (p68) (p70). This is the only known annexin that contains 8 (instead of 4) repeats. - Annexin VII (Synexin). - Annexin VIII (Vascular anticoagulant-beta) (VAC-beta). - Annexin IX from *Drosophila*. - Annexin X from *Drosophila*. - Annexin XI (Calcyclin-associated annexin) (CAP-50). - Annexin XII from *Hydra vulgaris*. - Annexin XIII (Intestine-specific annexin) (ISA). The signature pattern for this domain spans positions 9 to 61 of the repeat and includes the only perfectly conserved residue (an arginine in position 22)-

Consensus pattern: [TG]-[STV]-x(8)-[LIVMF]-x(2)-R-x(3)-[DEQNH]-x(7)-[IFY]-x(7)-[LIVMF]-x(3)-[LIVMF]-x(11)-[LIVMFA]-x(2)-[LIVMF]-

- [1] Raynal P., Pollard H.B. *Biochim. Biophys. Acta* 1197:63-93(1994).
- [2] Barton G.J., Newman R.H., Freemont P.S., Crumpton M.J. *Eur. J. Biochem.* 198:749-760(1991).
- [3] Burgoyne R.D., Geisow M.J. *Cell Calcium* 10:1-10(1989).
- [4] Haigler H.T., Fitch J.M., Jones J.M., Schlaepfer D.D. *Trends Biochem. Sci.* 14:48-50(1989).
- [5] Klee C.B. *Biochemistry* 27:6645-6653(1988).
- [6] Smith P.D., Moss S.E. *Trends Genet.* 10:241-246(1994).
- [7] Huber R., Roemisch J., Paques E.-P. *EMBO J.* 9:3867-3874(1990).
- [8] Fiedler K., Simons K. *Trends Biochem. Sci.* 20:177-178(1995).

57. (arf_1) ADP-ribosylation factors family signature

ADP-ribosylation factors (ARF) [1,2,3,4] are 20 Kd GTP-binding proteins involved in protein trafficking. They may modulate vesicle budding and uncoating within the Golgi apparatus. ARF's also act as allosteric activators of cholera toxin ADP-ribosyltransferase activity. They are evolutionary conserved and present in all eukaryotes. At least six forms of ARF are present in mammals and three in budding yeast. The ARF family also includes proteins highly related to ARF's but which lack the cholera toxin cofactor activity, they are collectively known as ARL's (ARF-like). ARD1 is a 64 Kd mammalian protein of unknown

biological function that contains an ARF domain at its C-terminal extremity. Proteins from the ARF family are generally included in the RAS 'superfamily' of small GTP-binding proteins [5], but they are only slightly related to the other RAS proteins. They also differ from RAS proteins in that they lack cysteine residues at their C-termini and are therefore not subject to prenylation. The ARFs are N-terminally myristoylated (the ARLs have not yet been shown to be modified in such a fashion). A conserved region in the C-terminal part of ARF's and ARL's has been selected as a signature pattern.

Consensus pattern: [HRQT]-x-[FYWI]-x-[LIVM]-x(4)-A-x(2)-G-x(2)-[LIVM]-x(2)-[GSA]-[LIVMF]-x-[WK]-[LIVM]-

Note: proteins belonging to this family also contain a copy of the ATP/GTP-binding motif 'A' (P-loop) (see <PDOC00017

[1] Boman A.L., Kahn R.A. Trends Biochem. Sci. 20:147-150(1995).

[2] Moss J., Vaughan M. Cell. Signal. 4:367-399(1993).

[3] Moss J., Vaughan M. Prog. Nucleic Acid Res. Mol. Biol. 45:47-65(1993).

[4] Amor J.C., Harrison D.H., Kahn R.A., Ringe D. Nature 372:704-708(1994).

[5] Valencia A., Chardin P., Wittinghofer A., Sander C. Biochemistry 30:4637-4648(1991).

20 (arf_2) ATP/GTP-binding site motif A (P-loop)

From sequence comparisons and crystallographic data analysis it has been shown [1,2,3,4,5,6] that an appreciable proportion of proteins that bind ATP or GTP share a number of more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region, which typically forms a flexible loop between a beta-strand and an alpha-helix.

- 25 This loop interacts with one of the phosphate groups of the nucleotide. This sequence motif is generally referred to as the 'A' consensus sequence [1] or the 'P-loop' [5]. There are numerous ATP- or GTP-binding proteins in which the P-loop is found. A number of protein families for which the relevance of the presence of such motif has been noted are listed below: - ATP synthase alpha and beta subunits (see <PDOC00137>). - Myosin heavy chains. - Kinesin heavy chains and kinesin-like proteins (see <PDOC00343>). - Dynamin and dynamin-like proteins (see <PDOC00362>). - Guanylate kinase (see <PDOC00670>). - Thymidine kinase (see <PDOC00524>). - Thymidylate kinase (see <PDOC01034>). - Shikimate kinase (see <PDOC00868>). - Nitrogenase iron protein family (nifH/frxC) (see <PDOC00580>). - ATP-binding proteins involved in 'active transport' (ABC transporters) [7] (see <PDOC00185>). -

DNA and RNA helicases [8,9,10]. - GTP-binding elongation factors (EF-Tu, EF-1alpha, EF-G, EF-2, etc.). - Ras family of GTP-binding proteins (Ras, Rho, Rab, Ral, Ypt1, SEC4, etc.). - Nuclear protein ran (see <PDOC00859>). - ADP-ribosylation factors family (see <PDOC00781>). - Bacterial dnaA protein (see <PDOC00771>). - Bacterial recA protein (see <PDOC00131>). - Bacterial recF protein (see <PDOC00539>). - Guanine nucleotide-binding proteins alpha subunits (Gi, Gs, Gt, G0, etc.). - DNA mismatch repair proteins mutS family (See <PDOC00388>). - Bacterial type II secretion system protein E (see <PDOC00567>). Not all ATP- or GTP-binding proteins are picked-up by this motif. A number of proteins escape detection because the structure of their ATP-binding site is completely different from that of the P-loop. Examples of such proteins are the E1-E2 ATPases or the glycolytic kinases. In other ATP- or GTP-binding proteins the flexible loop exists in a slightly different form; this is the case for tubulins or protein kinases. A special mention must be reserved for adenylate kinase, in which there is a single deviation from the P-loop pattern: in the last position Gly is found instead of Ser or Thr.

Consensus pattern: [AG]-x(4)-G-K-[ST]-

[1] Walker J.E., Saraste M., Runswick M.J., Gay N.J. EMBO J. 1:945-951(1982).

[2] Moller W., Amons R. FEBS Lett. 186:1-7(1985).

[3] Fry D.C., Kuby S.A., Mildvan A.S. Proc. Natl. Acad. Sci. U.S.A. 83:907-911(1986).

[4] Dever T.E., Glynias M.J., Merrick W.C. Proc. Natl. Acad. Sci. U.S.A. 84:1814-1818(1987).

[5] Saraste M., Sibbald P.R., Wittinghofer A. Trends Biochem. Sci. 15:430-434(1990).

[6] Koonin E.V. J. Mol. Biol. 229:1165-1174(1993).

[7] Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P. J. Bioenerg. Biomembr. 22:571-592(1990).

[8] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).

[9] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).

[10] Gorbalenya A.E., Koonin E.V., Donchenko A.P., Blinov V.M. Nucleic Acids Res. 17:4713-4730(1989).

The following enzymes have been shown [1] to be evolutionary related: - Arginase (EC 3.5.3.1), a ubiquitous enzyme which catalyzes the degradation of arginine to ornithine and urea [2]. - Agmatinase (EC 3.5.3.11) (agmatine ureohydrolase), a prokaryotic enzyme (gene *speB*) that catalyzes the hydrolysis of agmatine into putrescine and urea. -

- 5 Formiminoglutamase (EC 3.5.3.8) (formiminoglutamate hydrolase), a prokaryotic enzyme (gene *hutG*) that hydrolyzes N-formimino-glutamate into glutamate and formamide. - Hypothetical proteins from methanogenic archaeobacteria. These enzymes are proteins of about 300 amino-acid residues. Three conserved regions that contain charged residues which are involved in the binding of the two manganese ions [3] can be used as signature patterns. -

10

Consensus pattern: [LIVMF]-G-G-x-H-x-[LIVMT]-[STAV]-x-[PAG]-x(3)-[GSTA] [H binds manganese]-

Consensus pattern: [LIVM](2)-x-[LIVMFY]-D-[AS]-H-x-D [The two D's and the H bind manganese]-

- 15 Consensus pattern: [ST]-[LIVMFY]-D-[LIVM]-D-x(3)-[PAQ]-x(3)-P-[GSA]-x(7)-G [The two D's bind manganese]

[1] Ouzounis C., Kyripides N.C. J. Mol. Evol. 39:101-104(1994).

- [2] Jenkinson C.P., Grody W.W., Cederbaum S.D. Comp. Biochem. Physiol. 114B:107-132(196).

- 20 [3] Kanyo Z.F., Scolnick L.R., Ash D.E., Christianson D.W. Nature 383:554-557(1996).

59. (asp) Eukaryotic and viral aspartyl proteases active site

- 25 Aspartyl proteases, also known as acid proteases, (EC 3.4.23.-) are a widely distributed family of proteolytic enzymes [1,2,3] known to exist invertebrates, fungi, plants, retroviruses and some plant viruses. Aspartate proteases of eukaryotes are monomeric enzymes which consist of two domains. Each domain contains an active site centered on a catalytic aspartyl residue. The two domains most probably evolved from the duplication of an ancestral gene
- 30 encoding a primordial domain. Currently known eukaryotic aspartyl proteases are: - Vertebrate gastric pepsins A and C (also known as gastricsin). - Vertebrate chymosin (rennin), involved in digestion and used for making cheese. - Vertebrate lysosomal cathepsins D (EC 3.4.23.5) and E (EC 3.4.23.34). - Mammalian renin (EC 3.4.23.15) whose function is to generate angiotensin I from angiotensinogen in the plasma. - Fungal proteases such as

aspergillopepsin A (EC 3.4.23.18), candidapepsin (EC 3.4.23.24), mucoropepsin (EC 3.4.23.23) (mucor rennin), endothiapsin (EC 3.4.23.22), polyporopepsin (EC 3.4.23.29), and rhizopuspepsin (EC 3.4.23.21). - Yeast saccharopepsin (EC 3.4.23.25) (proteinase A) (gene PEP4). PEP4 is implicated in posttranslational regulation of vacuolar hydrolases. -

- 5 Yeast barrier pepsin (EC 3.4.23.35) (gene BAR1); a protease that cleaves alpha-factor and thus acts as an antagonist of the mating pheromone. - Fission yeast *ssa1* which is involved in degrading or processing the mating pheromones. Most retroviruses and some plant viruses, such as badnaviruses, encode for an aspartyl protease which is an homodimer of a chain of about 95 to 125 amino acids. In most retroviruses, the protease is encoded as a segment of
- 10 apolipoprotein which is cleaved during the maturation process of the virus. It is generally part of the pol polyprotein and, more rarely, of the gagpolyprotein. Conservation of the sequence around the two aspartates of eukaryotic aspartyl proteases and around the single active site of the viral proteases allows us to develop a single signature pattern for both groups of protease.

- 15 Consensus pattern: [LIVMFGAC]-[LIVMTADN]-[LIVFSA]-D-[ST]-G-[STAV]-[STAPDENQ]-x-[LIVMFSTNC]-x-[LIVMFGTA] [D is the active site residue]

Note: these proteins belong to families A1 and A2 in the classification of peptidases [4,E1

[1] Foltmann B. Essays Biochem. 17:52-84(1981).

- 20 [2] Davies D.R. Annu. Rev. Biophys. Chem. 19:189-215(1990).

[3] Rao J.K.M., Erickson J.W., Wlodawer A. Biochemistry 30:4663-4671(1991).

[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:105-120(1995).

- 25 60. (BIRA) Biotin repressor

[1] Wilson KP, Shewchuk LM, Brennan RG, Otsuka AJ, Matthews BW; Proc Natl Acad Sci USA 1992;89:9257-9261.

- 30 61. BTB/POZ domain

The BTB (for BR-C, tkk and bab) [1] or POZ (for Pox virus and Zinc finger)[2] domain is present near the N-terminus of a fraction of zinc finger

(zf-C2H2) proteins and in proteins that contain the Kelch motif

such as Kelch and a family of pox virus proteins. The BTB/POZ domain mediates homomeric dimerisation and in some instances heteromeric dimerisation [2]. The structure of the dimerised PLZF BTB/POZ domain has been solved and consists of a tightly intertwined homodimer. The central scaffolding of the protein is made up of a cluster of alpha-helices flanked by short beta-sheets at both the top and bottom of the molecule [3]. POZ domains from several zinc finger proteins have been shown to mediate transcriptional repression and to interact with components of histone deacetylase co-repressor complexes including N-CoR and SMRT [4,5,6]. The POZ or BTB domain is also known as BR-C/Ttk or ZiN

[1] Zollman S, Godt D, Prive GG, Couderc JL, Laski FA; Proc Natl Acad Sci U S A 1994;91:10717-10721.

[2] Bardwell VJ, Treisman R; Genes Dev 1994;8:1664-1677.

[3] Ahmad KF, Engel CK, Prive GG; Proc Natl Acad Sci U S A 1998;95:12123-12128.

[4] Deweindt C, Albagli O, Bernardin F, Dhordain P, Quief S,

Lantoine D, Kerckaert JP, Leprince D; Cell Growth Differ 1995;6:1495-1503.

[5] Huynh KD, Bardwell VJ; Oncogene 1998;17:2473-2484.

[6] Wong CW, Privalsky ML; J Biol Chem 1998;273:27695-27702.

62. (Bac GSPproteins) Bacterial type II secretion system protein D signature

A number of bacterial proteins, some of which are involved in a general secretion pathway (GSP) for the export of proteins (also called the type II pathway) [1 to 5], have been found to be evolutionary related. These proteins are listed below: - The 'D' protein from the GSP

operon of: *Aeromonas* (gene *exeD*); *Erwinia* (gene *outD*); *Escherichia coli* (gene *yheF*),

Klebsiella pneumoniae (gene *pulD*); *Pseudomonas aeruginosa* (gene *xcpQ*); *Vibrio cholerae*

(gene *epsD*) and *Xanthomonas campestris* (gene *xpsD*). - *comE* from *Haemophilus*

influenzae, involved in competence (DNA uptake). - *pilQ* from *Pseudomonas aeruginosa*,

which is essential for the formation of the pili. - *hofQ* (*hopQ*) from *Escherichia coli*. - *hrpH*

from *Pseudomonas syringae*, which is involved in the secretion of a proteinaceous elicitor of

the hypersensitivity response in plants. - *hrpA1* from *Xanthomonas campestris* pv.

vesicatoria, which is also involved in the hypersensitivity response. - *mxlD* from *Shigella*

flexneri which is involved in the secretion of the *Ipa* invasins which are necessary for

penetration of intestinal epithelial cells. - *omc* from *Neisseria gonorrhoeae*. - *yssC* from

Yersinia enterocolitica virulence plasmid pYV, which seems to be required for the export of

the Yop virulence proteins. - The gpIV protein from filamentous phages such as f1, ike, or m13. GpIV is said to be involved in phage assembly and morphogenesis. These proteins all seem to start with a signal sequence and are thought to be integral proteins in the outer membrane. As a signature pattern a conserved region in the C-terminal section of these

proteins has been selected

Consensus pattern: [GR]-[DEQKG]-[STVM]-[LIVMA](3)-[GA]-G-[LIVMFY]-x(11)-[LIVM]-P-[LIVMFYWGS]-[LIVMF]-[GSAE]-x-[LIVM]-P- [LIVMFYW](2)-x(2)-[LV]-F

[1] Salmond G.P.C., Reeves P.J. Trends Biochem. Sci. 18:7-12(1993).

[2] Reeves P.J., Whitcombe D., Wharam S., Gibson M., Allison G., Bunce N., Barallon R., Douglas P., Mulholland V., Stevens S., Walker S., Salmond G.P.C. Mol. Microbiol. 8:443-456(1993).

[3] Martin P.R., Hobbs M., Free P.D., Jeske Y., Mattick J.S. Mol. Microbiol. 9:857-868(1993).

[4] Hobbs M., Mattick J.S. Mol. Microbiol. 10:233-243(1993).

[5] Genin S., Boucher C.A. Mol. Gen. Genet. 243:112-118(1994).

63. (Bac globin) Protozoan/cyanobacterial globins signature

Globins are heme-containing proteins involved in binding and/or transporting oxygen [1]. Almost all globins belong to a large family (see <PDOC00793>), the only exceptions are the following proteins which form a family of their own[2,3]: - Monomeric hemoglobins from the protozoan Paramecium caudatum, Tetrahymena pyriformis and Tetrahymena thermophila. - Cyanoglobin from the cyanobacteria Nostoc commune. - Globins LI637 and LI410 from the chloroplast of the alga Chlamydomonas eugametos. - Mycobacterium tuberculosis hypothetical protein MtCY48.23. These proteins contain a conserved histidine which could be involved in heme-binding. As a signature pattern, a conserved region that ends with this residue was used

Consensus pattern: F-[LF]-x(5)-G-[PA]-x(4)-G-[KRA]-x-[LIVM]-x(3)-H-

[1] Concise Encyclopedia Biochemistry, Second Edition, Walter de Gruyter, Berlin New-York (1988).

[2] Takagi T. Curr. Opin. Struct. Biol. 3:413-418(1993).

[3] Couture M., Chamberland H., St-Pierre B., Lafontaine J., Guertin M.; Mol. Gen. Genet. 243:185-197(1994).

5

64. Band 7 protein family signature

Mammalian band 7 protein [1] (also known as 7.2B or stomatin) is an integral membrane phosphoprotein of red blood cells thought to regulate cation conductance by interacting with other proteins of the junctional complex of the membrane skeleton. Structurally, band 7 is evolutionary related to the following proteins: - *Caenorhabditis elegans* protein mec-2 [2]. Mec-2 positively regulates the activity of the putative mechanosensory transduction channel. It may links the mechanosensory channel and the microtubule cytoskeleton of the touch receptor neurons. - *Caenorhabditis elegans* proteins sto-1 to sto-4. - *Caenorhabditis elegans* protein unc-1. - *Escherichia coli* hypothetical protein ybbK. - *Mycobacterium tuberculosis* hypothetical protein MtCY277.09. - *Synechocystis* strain PCC 6803 hypothetical protein slr1128. - *Methanococcus jannaschii* hypothetical protein MJ0827. Structurally all these proteins consist of a short N-terminal domain which is followed by a transmembrane region and a variable size (from 170 to 350 residues) C-terminal domain. As a signature pattern, a conserved region located about 110 residues after the transmembrane domain was selected

20

Consensus pattern: R-x(2)-[LIV]-[SAN]-x(6)-[LIV]-D-x(2)-T-x(2)-W-G-[LIV]-[KRH]-[LIV]-x-[KR]-[LIV]-E-[LIV]-[KR]-

[1] Gallagher P.G., Forget B.G. *J. Biol. Chem.* 270:26358-26363(1995).

25 [2] Huang M., Gu G., Ferguson E.L., Chalfie M. *Nature* 378:292-295(1995).

65. Barwin domain signatures

Barwin [1] is a barley seed protein of 125 residues that binds weakly a chitin analog. It contains six cysteines involved in disulfide bonds, as shown in the following schematic representation.

30

```
+-----+ | ***** | *****
xxxxxxxxxxxxxxxxCxxxxxxxxCxxxxCxxxxxxxxCxxxxxxxxxxxxxxxxCxx ||| +-----
-----+ +-----+-----+ 'C': conserved cysteine involved in a disulfide bond. *':
```

position of the patterns. Barwin is closely related to the following proteins: - Hevein, a wound-induced protein found in the latex of rubber trees. - HEL, an *Arabidopsis thaliana* hevein-like protein [2]. - Win1 and win2, two wound-induced proteins from potato. - Pathogenesis-related protein 4 from tobacco. Hevein and the win1/2 proteins consist of an N-terminal chitin-binding domain followed by a barwin-like C-terminal domain. Barwin and its related proteins could be involved in a defense mechanism in plants. As signature patterns, two highly conserved regions that contain some of the cysteines were selected

Consensus pattern: C-G-[KR]-C-L-x-V-x-N [The two C's are involved in disulfide bonds]-

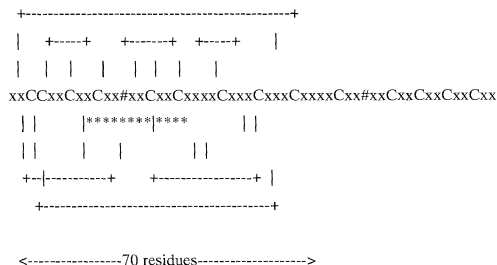
Consensus pattern: V-[DN]-Y-[EQ]-F-V-[DN]-C [C is involved in a disulfide bond]-

[1] Svensson B., Svendsen I., Hoejrup P., Roepstorff P., Ludvigsen S., Poulsen F.M. *Biochemistry* 31:8767-8770(1992).

[2] Potter S., Uknes S., Lawton K., Winter A.M., Chandler D., Dimaio J., Novitzky R., Ward E., Ryals J. *Mol. Plant Microbe Interact.* 6:680-685(1993).

66. (Bowman-Birk leg) Bowman-Birk serine protease inhibitors family signature

PROSITE cross-reference(s). The Bowman-Birk inhibitor family [1] is one of the numerous families of serine proteinase inhibitors. As it can be seen in the schematic representation, they have a duplicated structure and generally possess two distinct inhibitory sites:



'C': conserved cysteine involved in a disulfide bond.

#': active site residue.

*': position of the pattern.

These inhibitors are found in the seeds of all leguminous plants as well as in
 5 cereal grains. In cereals they exist in two forms, one of which is a
 duplication of the basic structure shown above [2]. The pattern that was developed
 to pick up sequences belonging to this family of inhibitors is in the central
 part of the domain and includes four cysteines.

- 10 Consensus pattern C-x(5,6)-[DENQKRHSTA]-C-[PASTDH]-[PASTDK]-[ASTDV]-C-
 [NDKS]-[DEKRHSTA]-C [The four C's are involved in disulfide bonds] Note this pattern
 can be found twice in some duplicated cereal inhibitors.

[1] Laskowski M., Kato I. Annu. Rev. Biochem. 49:593-626(1980).

- 15 [2] Tashiro M., Hashino K., Shiozaki M., Ibuki F., Maki Z. J. Biochem. 102:297-306(1987).

67. Pathogenesis-related protein Bet v I family signature

A number of plant proteins, which all seem to be involved in pathogen defense

- 20 response, are structurally related [1,2,3]. These proteins are:

- Bet v I, the major pollen allergen from white birch. Bet v I is the main cause of
 type I allergic reactions in Europe, North America and USSR.
- Aln g I, the major pollen allergen from alder.
- Api G I, the major allergen from celery.
- 25 - Car b I, the major pollen allergen from hornbeam.
- Cor a I, the major pollen allergen from hazel.
- Mal d I, the major pollen allergen from apple.
- Asparagus wound-induced protein AoPR1.
- Kidney bean pathogenesis-related proteins 1 and 2.
- 30 - Parsley pathogenesis-related proteins PR1-1 and PR1-3.
- Pea disease resistance response proteins pI49, pI176 and DRRG49-C.
- Pea abscisic acid-responsive proteins ABR17 and ABR18.
- Potato pathogenesis-related proteins STH-2 and STH-21.
- Soybean stress-induced protein SAM22.

These proteins are thought to be intracellularly located. They contain from 155 to 160 amino acid residues. As a signature pattern, a conserved region located in the third quarter of these proteins has been selected

Consensus pattern: G-x(2)-[LIVMF]-x(4)-E-x(2)-[CSTAEN]-x(8,9)-[GND]-G-[GS]-[CS]-
 5 x(2)-K-x(4)-[FY]-

[1] Breiteneder H., Pettenburger K., Bito A., Valenta R., Kraft D., Rumpold H., Scheiner O., Breitenbach M. *EMBO J.* 8:1935-1938(1989).

[2] Crowell D., John M.E., Russell D., Amasino R.M. *Plant Mol. Biol.* 18:459-466(1992).

10 [3] Warner S.A.J., Scott R., Draper J. *Plant Mol. Biol.* 19:555-561(1992).

68. bZIP transcription factors basic domain signature

The bZIP superfamily [1,2] of eukaryotic DNA-binding transcription factors groups together
 15 proteins that contain a basic region mediating sequence-specific DNA-binding followed by a leucine zipper required for dimerization. This family is quite large, therefore only a partial list of some representative members appears here. - Transcription factor AP-1, which binds selectively to enhancer elements in the cis control regions of SV40 and metallothionein IIA. AP-1, also known as c-jun, is the cellular homolog of the avian sarcoma virus 17 (ASV17)
 20 oncogene v-jun. - Jun-B and jun-D, probable transcription factors which are highly similar to jun/AP-1. - The fos protein, a proto-oncogene that forms a non-covalent dimer with c-jun. - The fos-related proteins fra-1, and fos B. - Mammalian cAMP response element (CRE) binding proteins CREB, CREM, ATF-1, ATF-3, ATF-4, ATF-5, ATF-6 and LRF-1. - Maize Opaque 2, a trans-acting transcriptional activator involved in the regulation of the production
 25 of zein proteins during endosperm. - Arabidopsis G-box binding factors GBF1 to GBF4, Parsley CPRF-1 to CPRF-3, Tobacco TAF-1 and wheat EMBP-1. All these proteins bind the G-box promoter elements of many plant genes. - Drosophila protein Giant, which represses the expression of both the kruppel and knirps segmentation gap genes. - Drosophila Box B binding factor 2 (BBF-2), a transcriptional activator that binds to fat body-specific enhancers
 30 of alcohol dehydrogenase and yolk protein genes. - Drosophila segmentation protein cap'n'collar (gene cnc), which is involved in head morphogenesis. - Caenorhabditis elegans skn-1, a developmental protein involved in the fate of ventral blastomeres in the early embryo. - Yeast GCN4 transcription factor, a component of the general control system that regulates the expression of amino acid-synthesizing enzymes in response to amino acid

starvation, and the related *Neurospora crassa* cpc-1 protein. - *Neurospora crassa* cys-3 which turns on the expression of structural genes which encode sulfur-catabolic enzymes. - Yeast MET28, a transcriptional activator of sulfur amino acids metabolism. - Yeast PDR4 (or YAP1), a transcriptional activator of the genes for some oxygen detoxification enzymes. -

5 Epstein-Barr virus trans-activator protein BZLF1.-

Consensus pattern: [KR]-x(1,3)-[RKSAQ]-N-x(2)-[SAQ](2)-x-[RKTAENQ]-x-R-x-[RK]-

[1] Hurst H.C. Protein Prof. 2:105-168(1995).[2] Ellenberger T. Curr. Opin. Struct. Biol.

10 4:12-21(1994).

69. Biotin-requiring enzymes attachment site

Biotin, which plays a catalytic role in some carboxyl transfer reactions, is covalently attached, via an amide bond, to a lysine residue in enzymes requiring this coenzyme [1,2,3,4]. Such enzymes are:

- Pyruvate carboxylase (EC 6.4.1.1).
- Acetyl-CoA carboxylase (EC 6.4.1.2).
- Propionyl-CoA carboxylase (EC 6.4.1.3).
- 20 - Methylcrotonyl-CoA carboxylase (EC 6.4.1.4).
- Geranoyl-CoA carboxylase (EC 6.4.1.5).
- Urea carboxylase (EC 6.3.4.6).
- Oxaloacetate decarboxylase (EC 4.1.1.3).
- Methylmalonyl-CoA decarboxylase (EC 4.1.1.41).
- 25 - Glutaconyl-CoA decarboxylase (EC 4.1.1.70).
- Methylmalonyl-CoA carboxyl-transferase (EC 2.1.3.1) (transcarboxylase).

Sequence data reveal that the region around the biocytin (biotin-lysine) residue is well conserved and can be used as a signature pattern.

30 Consensus pattern[GN]-[DEQTR]-x-[LIVMFY]-x(2)-[LIVM]-x-[AIV]-M-K-[LMAT]-x(3)-[LIVM]-x-[SAV] [K is the biotin attachment site] Note the domain around the biotin-binding lysine residue is evolutionary related to that around the lipoyl-binding lysine residue of 2-oxo acid dehydrogenase acyltransferases

- [1] Knowles J.R. Annu. Rev. Biochem. 58:195-221(1989).
- [2] Samols D., Thronton C.G., Murtif V.L., Kumar G.K., Haase F.C., Wood H.G. J. Biol. Chem. 263:6461-6464(1988).
- [3] Goss N.H., Wood H.G. Meth. Enzymol. 107:261-278(1984).
- 5 [4] Shenoy B.C., Xie Y., Park V.L., Kumar G.K., Beegen H., Wood H.G., Samols D. J. Biol. Chem. 267:18407-18412(1992).

2-oxo acid dehydrogenases acyltransferase component lipoyl binding site
The 2-oxo acid dehydrogenase multienzyme complexes [1,2] from bacterial and
10 eukaryotic sources catalyze the oxidative decarboxylation of 2-oxo acids to
the corresponding acyl-CoA. The three members of this family of multienzyme
complexes are:

- Pyruvate dehydrogenase complex (PDC).
- 2-oxoglutarate dehydrogenase complex (OGDC).
- 15 - Branched-chain 2-oxo acid dehydrogenase complex (BCOADC).

These three complexes share a common architecture: they are composed of
multiple copies of three component enzymes - E1, E2 and E3. E1 is a thiamine
pyrophosphate-dependent 2-oxo acid dehydrogenase, E2 a dihydrolipamide
acyltransferase, and E3 an FAD-containing dihydrolipamide dehydrogenase.

20 E2 acyltransferases have an essential cofactor, lipoyl acid, which is
covalently bound via an amide linkage to a lysine group. The E2 components of
OGDC and BCOADC bind a single lipoyl group, while those of PDC bind either one
(in yeast and in *Bacillus*), two (in mammals), or three (in *Azotobacter* and in
Escherichia coli) lipoyl groups [3].

25 In addition to the E2 components of the three enzymatic complexes described
above, a lipoyl acid cofactor is also found in the following proteins:

- H-protein of the glycine cleavage system (GCS) [4]. GCS is a multienzyme
complex of four protein components, which catalyzes the degradation of
glycine. H protein shuttles the methylamine group of glycine from the P
30 protein to the T protein. H-protein from either prokaryotes or eukaryotes
binds a single lipoyl group.
- Mammalian and yeast pyruvate dehydrogenase complexes differ from that of
other sources, in that they contain, in small amounts, a protein of unknown
function - designated protein X or component X. Its sequence is closely

related to that of E2 subunits and seems to bind a lipoiic group [5].

- Fast migrating protein (FMP) (gene acoC) from *Alcaligenes eutrophus* [6].

This protein is most probably a dihydrolipamide acyltransferase involved in acetoin metabolism.

- 5 A signature pattern was developed which allows the detection of the lipoyl-binding site.

Consensus pattern[GN]-x(2)-[LIVF]-x(5)-[LIVFC]-x(2)-[LIVFA]-x(3)-K-[STAIV]-[STAVQDN]-x(2)-[LIVMFS]-x(5)-[GCN]-x-[LIVMFY] [K is the lipoyl-binding site] Note
10 the domain around the lipoyl-binding lysine residue is evolutionary related to that around the biotin-binding lysine residue of biotin requiring enzymes

[1] Yeaman S.J. Biochem. J. 257:625-632(1989).

[2] Yeaman S.J. Trends Biochem. Sci. 11:293-296(1986).

- 15 [3] Russel G.C., Guest J.R. Biochim. Biophys. Acta 1076:225-232(1991).

[4] Fujiwara K., Okamura-Ikeda K., Motokawa Y. J. Biol. Chem. 261:8836-8841(1986).

[5] Behal R.H., Browning K.S., Hall T.B., Reed L.J. Proc. Natl. Acad. Sci. U.S.A. 86:8732-8736(1989).

[6] Priefert H., Hein S., Krueger N., Zeh K., Schmidt B., Steinbuechel A. J. Bacteriol.

- 20 173:4056-4071(1991).

70. C2 (C2 domain) Number of members: 295

Some isozymes of protein kinase C (PKC) [1,2] contain a domain, known as C2, of about
25 116 amino-acid residues which is located between the two copies of the C1 domain (that bind phorbol esters and diacylglycerol) (see <PDOC00379>) and the protein kinase catalytic domain (see <PDOC00100>). Regions with significant homology [3,E1] to the C2-domain have been found in the following proteins:

- PKC isoforms alpha, beta and gamma and *Drosophila* isoforms PKC1 and PKC2.
- 30 - PKC isoforms delta, epsilon and eta, *Caenorhabditis elegans* kin-13 and yeast PKC1 have a C2-like domain at the N-terminal extremity [4].
- Yeast cAMP dependent protein kinase SCH9 contains a C2-like domain.

- Mammalian phosphatidylinositol-specific phospholipase C (PI-PLC) (see <PDOC50007>) isoforms beta, gamma and delta as well as several non-mammalian PI-PLCs have a C2-like domain C-terminal of the catalytic domain.

5 - Mammalian and plants phosphatidylinositol-3-kinase have a C2-like domain in the central region of the 110 Kd catalytic subunit.

- Yeast phosphatidylserine-decarboxylase 2 (gene PSD2) contains a C2 domain in its central region.

- Cytosolic phospholipase D from plants and cytosolic phospholipase A2 have a C2-like domain at their N-terminus.

10 - Synaptotagmins (p65). This is a family of related synaptic vesicle proteins that bind acidic phospholipids and that may have a regulatory role in the membrane interactions during trafficking of synaptic vesicles at the active zone of the synapse. All isoforms of synaptotagmins have two copies of the C2 domain in their C-terminal region.

- Rabphilin-3A, a synaptic protein contains two C2 domains.

15 - Caenorhabditis elegans protein unc-13 whose function is not known. Unc-13 has a C2 domain in its central part and a C2-like domain at the C-terminus.

- rasGAP and the breakpoint cluster protein bcr have a C2-domain C-terminal of a PH-domain.

- Yeast protein BUD2 (or CLA2) has a C2-domain in the central region.

20 - Yeast protein RSP5 and human protein NEDD-4, both proteins also contain WW domains (see <PDOC50020>).

- Perforin (see <PDOC00251>) has a C2 domain at the C-terminus. It is the only extracellular protein known to contain a C2 domain.

- Yeast hypothetical protein YML072C has a C2 domain.

25 - Yeast hypothetical protein YNL087W has three C2 domains.

- Caenorhabditis elegans hypothetical protein F37A4.7 has two C2 domains.

The C2 domain is thought to be involved in calcium-dependent phospholipid binding [5].

Since domains related to the C2 domain are also found in proteins that do not bind calcium,

other putative functions for the C2 domain like e.g. binding to inositol-1,3,4,5-tetrakisphosphate

30 have been suggested [6]. Recently, the 3D structure of the first C2 domain of

synaptotagmin has been reported [7], the domain forms an eight-stranded beta sandwich. The

signature pattern that has been developed for the C2 domain is located in a conserved part of

that domain, the connecting loop between beta strands 2 and 3. A profile has been

developed for the C2 domain that covers the total domain.

-Consensus pattern: [ACG]-x(2)-L-x(2,3)-D-x(1,2)-[NGSTLIF]-[GTMR]-x-[STAP]-D-[PA]-[FY]

-Note: this documentation entry is linked to both a signature pattern and a profile. As the profile is much more sensitive than the pattern, you should use it if you have access to the necessary software tools to do so.

[1]Medline: 96367095 Extending the C2 domain family: C2s in PKCs delta, epsilon, eta and theta, phospholipases, GAPs and perforin. Ponting CP, Parker PJ; Protein Sci 1996;5:162-166.

[1] Azzi A., Boscoboinik D., Hensey C. Eur. J. Biochem. 208:547-557(1992).

[2] Stabel S. Semin. Cancer Biol. 5:277-284(1994).

[3] Brose N., Hofmann K.O., Hata Y., Suedhof T.C. J. Biol. Chem. 270:25273-25280(1995).

[4] Sossin W.S., Schwartz J.H. Trends Biochem. Sci. 18:207-208(1993).

[5] Davletov B.A., Suedhof T.C. J. Biol. Chem. 268:26386-26390(1993).

[6] Fukuda M., Aruga J., Niinobe M., Aimoto S., Mikoshiba K. J. Biol. Chem. 269:29206-29211(1994).

[6] Sutton R.B., Davletov B.A., Berghuis A.M., Suedhof T.C., Sprang S.R. Cell 80:929-938(1995).

71. CAP (CAP protein) Number of members: 11

In budding and fission yeasts the CAP protein is a bifunctional protein whose N-terminal domain binds to adenylyl cyclase, thereby enabling that enzyme to be activated by upstream regulatory signals, such as Ras. The function of the C-terminal domain is less clear, but it is required for normal cellular morphology and growth control [1]. CAP is conserved in higher eukaryotic organisms where its function is not yet clear [2].

Structurally, CAP is a protein of 474 to 551 residues which consist of two domains separated by a proline-rich hinge. Two signature patterns, one corresponding to a conserved region in the N-terminal extremity and the other to a C-terminal region have been developed.

-Consensus pattern: [LIVM](2)-x-R-L-[DE]-x(4)-R-L-E

-Consensus pattern: D-[LIVMFY]-x-E-x-[PA]-x-P-E-Q-[LIVMFY]-K

[1] Kawamukai M., Gerst J., Field J., Riggs M., Rodgers L., Wigler M., Young D. Mol. Biol. Cell 3:167-180(1992).

[2] Yu G., Swiston J., Young D. J. Cell Sci. 107:1671-1678(1994).

5

72. CAP_GLY (CAP-Gly domain)

CAP stands for cytoskeleton-associated proteins. Swiss:P39937 may be a member but has not been included. It has a weak match to the family between residues 22-67. Number of members: 24

10

[1]Medline: 93242656. Sequence homologies between four cytoskeleton-associated proteins. Riehemann K, Sorg C; Trends Biochem Sci 1993;18:82-83.

It has been shown [1] that some cytoskeleton-associated proteins (CAP) share the presence of a conserved, glycine-rich domain of about 42 residues, called here CAP-Gly. Proteins known to contain this domain are listed below.

15

- Restin (also known as cytoplasmic linker protein-170 or CLIP-170), a 160 Kd protein associated with intermediate filaments and that links endocytic vesicles to microtubules. Restin contains two copies of the CAP-Gly domain.

20

- Vertebrate dynactin (150 Kd dynein-associated polypeptide; DAP) and Drosophila glued, a major component of activator I, a 20S polypeptide complex that stimulates dynein-mediated vesicle transport.

- Yeast protein BIK1 which seems to be required for the formation or stabilization of microtubules during mitosis and for spindle pole body fusion during conjugation.

25

- Yeast protein NIP100 (NIP80).

- Human protein CKAP1/TFCB, Schizosaccharomyces pombe protein alp11 and Caenorhabditis elegans hypothetical protein F53F4.3. These proteins contain a N-terminal ubiquitin domain (see <PDOC00271>) and a C-terminal CAP-Gly domain.

- Caenorhabditis elegans hypothetical protein M01A8.2.

30

- Yeast hypothetical protein YNL148c.

Structurally, these proteins are made of three distinct parts: an N-terminal section that is most probably globular and contains the CAP-Gly domain, a large central region predicted to be in an alpha-helical coiled-coil conformation and, finally, a short C-

terminal globular domain. The signature for the CAP-Gly domain corresponds to the first 32 residues of the domain and includes five of the six conserved glycines.

-Consensus pattern: G-x(8,10)-[FYW]-x-G-[LIVM]-x-[LIVMFY]-x(4)-G-K-[NH]-x-G-

5 [STAR]-x(2)-G-x(2)-[LY]-F

[1] Riehemann K., Sorg C. Trends Biochem. Sci. 18:82-83(1993).

10 73. (CBD 1)

Cellulose-binding domain, fungal type

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1].

15

Structurally, cellulases and xylanases generally consist of a catalytic domain joined to a cellulose-binding domain (CBD) by a short linker sequence rich in proline and/or hydroxy-amino acids.

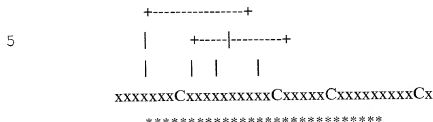
20

The CBD of a number of fungal cellulases has been shown to consist of 36 amino acid residues. Enzymes known to contain such a domain are:

- Endoglucanase I (gene egl1) from *Trichoderma reesei*.
- Endoglucanase II (gene egl2) from *Trichoderma reesei*.
- 25 - Endoglucanase V (gene egl5) from *Trichoderma reesei*.
- Exocellobiohydrolase I (gene CBHI) from *Humicola grisea*, *Neurospora crassa*, *Phanerochaete chrysosporium*, *Trichoderma reesei*, and *Trichoderma viride*.
- Exocellobiohydrolase II (gene CBHII) from *Trichoderma reesei*.
- Exocellobiohydrolase 3 (gene cel3) from *Agaricus bisporus*
- 30 - Endoglucanases B, C2, F and K from *Fusarium oxysporum*.

The CBD domain is found either at the N-terminal (Cbh-II or egl2) or at the C-terminal extremity (Cbh-I, egl1 or egl5) of these enzymes. As it is shown in the following schematic

representation, there are four conserved cysteines in this type of CBD domain, all involved in disulfide bonds.



10 'C': conserved cysteine involved in a disulfide bond.

'*': position of the pattern.

Such a domain has also been found in a putative polysaccharide binding protein from the red alga, *Porphyra purpurea* [2]. Structurally, this protein consists of four tandem repeats of the CBD domain.

Consensus pattern C-G-G-x(4,7)-G-x(3)-C-x(5)-C-x(3,5)-[NHG]-x-[FYWM]-x(2)-Q-C [The four C's are involved in disulfide bonds] Sequences known to belong to this class detected by the pattern ALL.

[1] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).

[2] Liu Q., der Meer J.P., Reith M.E.

74. CBS domain. 3D Structure found as a subdomain in TIM barrel of inosine-. CBS domain web page. CBS domains are small intracellular modules mostly found in 2 or four copies within a protein. CBS domains are found in cystathionine-beta-synthase (CBS) where mutations lead to homocystinuria. Two CBS domains are found in inosine-monophosphate dehydrogenase from all species, however the CBS domains are not needed for activity. Two CBS domains are found in intracellular loops of several chloride channels. Mutations in this domain of Swiss:P35520 lead to homocystinuria.

Number of members: 414

[1]Medline: 97172695 The structure of a domain common to archaeobacteria and the homocystinuria disease protein. Bateman A; Trends Biochem Sci 1997;22:12-13.

[2]Medline: 96279836 Structure and mechanism of inosine monophosphate dehydrogenase in complex with the immunosuppressant mycophenolic-acid. Sintchak MD, Fleming MA,

5 Futer O, Raybuck SA, Chambers SP, Caron PR, Murcko MA, Wilson KP; Cell 1996;85:921-930.

Discovery of CBS domain.

[3]Medline: 97259972 CBS domains in CIC chloride channels implicated in myotonia and nephrolithiasis (kidney stones). Ponting CP; J Mol Med 1997;75:160-163.

10

75. CDP-OH_P_transf (CDP-alcohol phosphatidyltransferase)

All of these members have the ability to catalyze the displacement of CMP from a CDP-alcohol by a second alcohol with formation of a phosphodiester bond and concomitant breaking of a phosphoride anhydride bond. Number of members: 32

15

A number of phosphatidyltransferases, which are all involved in phospholipid biosynthesis and that share the property of catalyzing the displacement of CMP from a CDP-alcohol by a second alcohol with formation of a phosphodiester bond and concomitant breaking of a phosphoride anhydride bond share a conserved sequence region [1,2]. These enzymes are:

20

- Ethanolaminephosphotransferase (EC 2.7.8.1) from yeast (gene EPT1).
- Diacylglycerol cholinephosphotransferase (EC 2.7.8.2) from yeast (gene CPT1).
- Phosphatidylglycerophosphate synthase (EC 2.7.8.5) (CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase) from bacteria (gene pgsA).
- Phosphatidylserine synthase (EC 2.7.8.8) (CDP-diacylglycerol--serine O-phosphatidyltransferase) from yeast (gene CHO1) and from *Bacillus subtilis* (gene pssA).
- 25 - Phosphatidylinositol synthase (EC 2.7.8.11) (CDP-diacylglycerol--inositol 3-phosphatidyltransferase) from yeast (gene PIS).

30

These enzymes are proteins of from 200 to 400 amino acid residues. The conserved region contains three aspartic acid residues and is located in the N-terminal section of the sequences.

-Consensus pattern: D-G-x(2)-A-R-x(8)-G-x(3)-D-x(3)-D

[1]Medline: 97075020 Two-dimensional 1H-NMR of transmembrane peptides from Escherichia coli phosphatidylglycerophosphate synthase in micelles. Morein S, Trouard TP, Hauksson JB, Rilfors L, Arvidson G, Lindblom G; Eur J Biochem 1996;241:489-497.

[1] Nikawa J.-I., Kodaki T., Yamashita S.

5 J. Biol. Chem. 262:4876-4881(1987).

[2] Hjelmstad R.H., Bell R.M.

J. Biol. Chem. 266:5094-5134(1991).

10 76. CHOD (Cholesterol oxidase) Members of the GMC oxidoreductase family. Number of members: 3

[1]Medline: 94032271. Crystal structure of cholesterol oxidase complexed with a steroid substrate: implications for flavin adenine dinucleotide dependent alcohol oxidases. Li J,

15 Vrieling A, Brick P, Blow DM; Biochemistry 1993;32:11507-11515.

The following FAD flavoproteins oxidoreductases have been found [1,2] to be evolutionary related. These enzymes, which are called 'GMC oxidoreductases', are listed below.

- Glucose oxidase (EC 1.1.3.4) (GOX) from Aspergillus niger. Reaction catalyzed: glucose

20 + oxygen -> delta-luconolactone + hydrogen peroxide.

- Methanol oxidase (EC 1.1.3.13) (MOX) from fungi. Reaction catalyzed: methanol +

oxygen -> acetaldehyde + hydrogen peroxide.

- Choline dehydrogenase (EC 1.1.99.1) (CHD) from bacteria. Reaction catalyzed: choline + unknown acceptor -> betaine acetaldehyde + reduced acceptor.

25 - Glucose dehydrogenase (GLD) (EC 1.1.99.10) from Drosophila. Reaction catalyzed: glucose + unknown acceptor -> delta-gluconolactone + reduced acceptor.

- Cholesterol oxidase (CHOD) (EC 1.1.3.6) from Brevibacterium sterolicum and Streptomyces strain SA-COO. Reaction catalyzed: cholesterol + oxygen -> cholest-4-en-3-one + hydrogen peroxide.

30 - AlkJ [3], an alcohol dehydrogenase from Pseudomonas oleovorans, which converts aliphatic medium-chain-length alcohols into aldehydes. This family also includes a lyase:

- (R)-mandelonitrile lyase (EC 4.1.2.10) (hydroxynitrile lyase) from plants [4], an enzyme involved in cyanogenesis, the release of hydrogen cyanide from injured tissues.

These enzymes are proteins of size ranging from 556 (CHD) to 664 (MOX) amino acid residues which share a number of regions of sequence similarities. One of these regions, located in the N-terminal section, corresponds to the FAD ADP- binding domain. The function of the other conserved domains is not yet known; two of these domains have been selected as signature patterns. The first one is located in the N-terminal section of these enzymes, about 50 residues after the ADP-binding domain, while the second one is located in the central section.

-Consensus pattern: [GA]-[RKN]-x-[LIV]-G(2)-[GST](2)-x-[LIVM]-N-x(3)-[FYWA]- x(2)-[PAG]-x(5)-[DNESH]

-Consensus pattern: [GS]-[PSTA]-x(2)-[ST]-P-x-[LIVM](2)-x(2)-S-G-[LIVM]-G

[1] Cavener D.R. J. Mol. Biol. 223:811-814(1992).

[2] Henikoff S., Henikoff J.G. Genomics 19:97-107(1994).

[3] van Beilen J.B., Eggink G., Enequist H., Bos R., Witholt B. Mol. Microbiol. 6:3121-3136(1992).

[4] Cheng I.P., Poulton J.E. Plant Cell Physiol. 34:1139-1143(1993).

77. CKS (Cyclin-dependent kinase regulatory subunit) Number of members: 11. Cyclin-dependent kinases (CDK) are protein kinases which associate with cyclins to regulate eukaryotic cell cycle progression. The most well known CDK is p34-cdc2 (CDC28 in yeast, which is required for entry into S-phase and mitosis. CDK's bind to a regulatory subunit which is essential for their biological function. This regulatory subunit is a small protein of 79 to 150 residues. In yeast (gene CKS1) and in fission yeast (gene suc1) a single isoform is known, while mammals have two highly related isoforms. It has been shown [1] that these CDK regulatory subunits assemble as an hexamer which then acts as a hub for the oligomerization of six CDK catalytic subunits. The sequence of CDK regulatory subunits are highly conserved therefore, the two most conserved regions have been used as signature patterns.

-Consensus pattern: Y-S-x-[KR]-Y-x-[DE](2)-x-[FY]-E-Y-R-H-V-x-[LV]-[PT]-[KRP]

-Consensus pattern: H-x-P-E-x-H-[IV]-L-L-F-[KR]

[1] Parge H.E., Arvai A.S., Murtari D.J., Reed S.I., Tainer J.A. Science 262:387-395(1993).

78. CK_II_beta (Casein kinase II regulatory subunit)

- 5 Number of members: 16. Casein kinase II (CK-2) [1] is an ubiquitous eukaryotic serine/threonine protein kinase which is found both in the cytoplasm and the nucleus and whose substrates are numerous. It generally phosphorylates Ser or Thr at the N-terminal of stretch of acidic residues (see <PDOC00006>). CK-2 exists as an heterotetramer composed of two catalytic subunits (alpha) and two regulatory subunits (beta). In most
- 10 species there are two closely related isoforms of the catalytic subunit: alpha and alpha'. Some species, such as fungi and plants, express two forms of regulatory subunits: beta and beta'. The exact function of the regulatory subunit is not yet known. It is a highly conserved protein of about 25 Kd that contains, in its central section, a cysteine-rich motif that could be involved in binding a metal such as zinc [2]. This region has been used as a signature
- 15 pattern.

-Consensus pattern: C-P-x-[LIVMY]-x-C-x(5)-[LI]-P-[LIVMC]-G-x(9)-V-[KR]-x(2)-C-P-x-C

- 20 [1] Allende J.E., Allende C.C. FASEB J. 9:313-323(1995).
[2] Reed J.C., Bidwai A.P., Glover C.V.C. J. Biol. Chem. 269:18192-18200(1994).

79. CLP_protease (Clp protease)

- 25 These proteins belong to family S14 in the classification of peptidases.
- !- The Clp protease has an active site catalytic triad. In E. coli Clp protease, ser-111, his-136 and asp-185 form the catalytic triad.
- !- Swiss:P48254 has lost all of these active site residues and is therefore inactive.
- !- Swiss:P42379 contains two large insertions, Swiss:P42380 contains one large insertion.
- 30 Number of members: 38
- The endopeptidase Clp (EC 3.4.21.92) from Escherichia coli cleaves peptides in various proteins in a process that requires ATP hydrolysis [1,2]. Clp is a dimeric protein which consists of a proteolytic subunit (gene clpP) and either of two related ATP-binding regulatory subunits (genes clpA and clpX). ClpP is a serine protease which has a chymotrypsin-like

activity. Its catalytic activity seems to be provided by a charge relay system similar to that of the trypsin family of serine proteases, but which evolved by independent convergent evolution. Proteases highly similar to ClpP have been found to be encoded in the genome of the chloroplast of plants and seem to be also present in other eukaryotes. The sequences around two of the residues involved in the catalytic triad (a serine and a histidine) are highly conserved and can be used as signature patterns specific to that category of proteases.

-Consensus pattern: T-x(2)-[LIVMF]-G-x-A-[SAC]-S-[MSA]-[PAG]-[STA] [S is the active site residue]

-Consensus pattern: R-x(3)-[EAP]-x(3)-[LIVMFYT]-M-[LIVM]-H-Q-P [H is the active site residue]

[1]Medline: 98050920. The structure of ClpP at 2.3 angstroms resolution suggests a model for ATP-dependent proteolysis. Wang J, Hartling JA, Flanagan JM; Cell 1997;91:447-456.

[1] Maurizi M.R., Clark W.P., Kim S.-H., Gottesman S. J. Biol. Chem. 265:12546-12552(1990).

[2] Gottesman S., Maurizi M.R. Microbiol. Rev. 56:592-621(1992).

[3] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

80. CNG_membrane (Transmembrane region cyclic Nucleotide Gated Channel)

[1]Medline: 94224763. Cyclic nucleotide-gated channels: an expanding new family of ion channels. Yau KW; Proc Natl Acad Sci USA 1994;91:3481-3483.

This family is found to the N-terminus of the cNMP_binding. Number of members: 56. Proteins that bind cyclic nucleotides (cAMP or cGMP) share a structural domain of about 120 residues [1-3]. The best studied of these proteins is the prokaryotic catabolite gene activator (also known as the cAMP receptor protein) (gene crp) where such a domain is known to be composed of three alpha-helices and a distinctive eight-stranded, antiparallel beta-barrel structure. Such a domain is known to exist in the following proteins:

- Prokaryotic catabolite gene activator protein (CAP).

- cAMP- and cGMP-dependent protein kinases (cAPK and cGPK). Both types of kinases contains two tandem copies of the cyclic nucleotide-binding domain. The cAPK's are composed of two different subunits: a catalytic chain and a regulatory chain which contains

both copies of the domain. The cGPK's are single chain enzymes that include the two copies of the domain in their N-terminal section. The nucleotide specificity of cAPK and cGPK is due to an amino acid in the conserved region of beta-barrel 7: a threonine that is invariant in cGPK is an alanine in most cAPK.

5 - Vertebrate cyclic nucleotide-gated ion-channels. Two such cations channels have been fully characterized. One is found in rod cells where it plays a role in visual signal transduction. It specifically binds to cGMP leading to an opening of the channel and thereby causing a depolarization of rod photoreceptors. In olfactory epithelium a similar, cAMP-binding, channel plays a role in odorant signal transduction. There are six invariant
10 amino acids in this domain, three of which are glycine residues that are thought to be essential for maintenance of the structural integrity of the beta-barrel. Two signature patterns have been developed for this domain. The first pattern is located within beta-barrels and 3 and contains the first two conserved Gly. The second pattern is located within beta-barrels 6 and 7 and contains the third conserved Gly as well as the three other invariant
15 residues.

-Consensus pattern: [LIVM]-[VIC]-x(2)-G-[DENQTA]-x-[GAC]-x(2)-[LIVMFY](4)-x(2)-G

-Consensus pattern: [LIVMF]-G-E-x-[GAS]-[LIVM]-x(5,11)-R-[STAQ]-A-x-[LIVMA]-x-[STACV]

[1] Weber I.T., Shabb J.B., Corbin J.D. Biochemistry 28:6122-6127(1989).

[2] Kaupp U.B. Trends Neurosci. 14:150-157(1991).

[3] Shabb J.B., Corbin J.D. J. Biol. Chem. 267:5723-5726(1992).

81. COX10_ctaB_cyoE (Cytochrome c oxidase assembly factor)

[1]Medline: 95191390

Biosynthesis and functional role of haem O and haem A

Mogi T, Saiki K, Anraku Y; Mol Microbiol 1994;14:391-398.

30 Cytochrome c oxidase is a multi subunit enzyme. The complexity of this enzyme requires assistance in building the complex.

This is carried out by the Cytochrome c oxidase assembly factor.

Number of members: 31

Cytochrome c oxidase is an oligomeric enzymatic complex which seems to require the aid of a number of proteins that either act as chaperonins to help the subunits of the enzyme to fold correctly, or assist in the assembly of the metal centers [1]. One of these subunits is known as COX10 in yeast and as ctaB [2] in aerobic prokaryotes. It is evolutionary related to cyoE protein from the Escherichia coli cytochrome O terminal oxidase complex.

These proteins probably contain [3] seven transmembrane segments. The most conserved region is located in a loop between the second and third of these segments and has been selected as a signature pattern.

-Consensus pattern: [ED]-x-D-x(2)-M-x-R-T-x(2)-R-x(4)-G

[1] Nobrega M.P., Nobrega F.G., Tzagoloff A.

J. Biol. Chem. 265:14220-14226(1990).

[2] Cao J., Hosler J., Shapleigh J., Revzin A., Ferguson-Miller S.

J. Biol. Chem. 267:24273-24278(1992).

[3] Chepuri V., Gennis R.B.

J. Biol. Chem. 265:12978-12986(1990).

82. COX3 (Cytochrome c oxidase subunit III)

This family corresponds to chains c and p.

[1]Medline: 96216288

The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 Å. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S; Science 1996;272:1136-1144.
Number of members: 224

83. COX5B (Cytochrome c oxidase subunit Vb)

[1]

Medline: 96216288

The whole structure of the 13-subunit oxidized cytochrome c

oxidase at 2.8 A.

Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H,
Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S;

Science 1996;272:1136-1144.

- 5 This family consists of chains F and S

Number of members: 10

- Cytochrome c oxidase (EC 1.9.3.1) [1] is an oligomeric enzymatic complex which
is a component of the respiratory chain complex and is involved in the
10 transfer of electrons from cytochrome c to oxygen. In eukaryotes this enzyme
complex is located in the mitochondrial inner membrane; in aerobic prokaryotes
it is found in the plasma membrane. In addition to the three large subunits
that form the catalytic center of the enzyme complex there are, in eukaryotes,
a variable number of small polypeptidic subunits. One of these subunits which
15 is known as Vb in mammals, V in slime mold and IV in yeast, binds a zinc atom.
The sequence of subunit Vb is well conserved and includes three conserved
cysteines that are thought to coordinate the zinc ion [2]. Two of these
cysteines are clustered in the C-terminal section of the subunit; this region has been selected
as a signature pattern.

- 20 -Consensus pattern: [LIVM](2)-[FYW]-x(10)-C-x(2)-C-G-x(2)-[FY]-K-L [The two C's
probably bind zinc]

[1] Capaldi R.A., Malatesta F., Darley-USmar V.M.

- 25 Biochim. Biophys. Acta 726:135-148(1983).

[2] Rizzuto R., Sandona D., Brini M., Capaldi R.A., Bisson R.

Biochim. Biophys. Acta 1129:100-104(1991).

- 30 84. COesterase (Carboxylesterases)

Cholinesterase pages

The prints entry is specific to acetylcholinesterase

Number of members: 273

Higher eukaryotes have many distinct esterases. Among the different types are those which act on carboxylic esters (EC 3.1.1.-). Carboxyl-esterases have been classified into three categories (A, B and C) on the basis of differential patterns of inhibition by organophosphates. The sequence of a number of type-B carboxylesterases indicates [1,2,3] that the majority are evolutionary related. This family currently consists of the following proteins:

- Acetylcholinesterase (EC 3.1.1.7) (AChE) [E1] from vertebrates and from *Drosophila*.

- Mammalian cholinesterase II (butyryl cholinesterase) (EC 3.1.1.8).

Acetylcholinesterase and cholinesterase II are closely related enzymes that hydrolyze choline esters [4].

- Mammalian liver microsomal carboxylesterases (EC 3.1.1.1).

- *Drosophila* esterase 6, produced in the anterior ejaculatory duct of the male insect reproductive system where it plays an important role in its reproductive biology.

- *Drosophila* esterase P.

- *Culex pipiens* (mosquito) esterases B1 and B2.

- *Myzus persicae* (peach-potato aphid) esterases E4 and FE4.

- Mammalian bile-salt-activated lipase (BAL) [5], a multifunctional lipase which catalyzes fat and vitamin absorption. It is activated by bile salts in infant intestine where it helps to digest milk fats.

- Insect juvenile hormone esterase (JH esterase) (EC 3.1.1.59).

- Lipases (EC 3.1.1.3) from the fungi *Geotrichum candidum* and *Candida rugosa*.

- *Caenorhabditis* gut esterase (gene ges-1).

- Duck fatty acyl-CoA hydrolase, medium chain (EC 3.1.2.14), an enzyme that may be associated with peroxisome proliferation and may play a role in the production of 3-hydroxy fatty acid diester pheromones.

- Membrane enclosed crystal proteins from slime mold. These proteins are, most probably esterases; the vesicles where they are found have therefore been termed esterosomes.

So far two bacterial proteins have been found to belong to this family:

- Phenmedipham hydrolase (phenylcarbamate hydrolase), an *Arthrobacter* oxidans plasmid-encoded enzyme (gene *pcd*) that degrades the phenylcarbamate herbicides phenmedipham and desmedipham by hydrolyzing their central carbamate linkages.
- Para-nitrobenzyl esterase from *Bacillus subtilis* (gene *pnbA*).

The following proteins, while having lost their catalytic activity, contain a domain evolutionary related to that of carboxylesterases type-B:

- Thyroglobulin (TG), a glycoprotein specific to the thyroid gland, which is the precursor of the iodinated thyroid hormones thyroxine (T4) and triiodo thyronine (T3).
- Drosophila protein neuractin (gene *nrt*) which may mediate or modulate cell adhesion between embryonic cells during development.
- Drosophila protein glutactin (gene *gl*t), whose function is not known.

As is the case for lipases and serine proteases, the catalytic apparatus of esterases involves three residues (catalytic triad): a serine, a glutamate or aspartate and a histidine. The sequence around the active site serine is well conserved and can be used as a signature pattern. A conserved region located in the N-terminal section containing a cysteine involved in a disulfide bond has been selected as a second signature pattern.

- Consensus pattern: F-[GR]-G-x(4)-[LIVM]-x-[LIV]-x-G-x-S-[STAG]-G[S is the active site residue]
- Consensus pattern: [ED]-D-C-L-[YT]-[LIV]-[DNS]-[LIV]-[LIVFYW]-x-[PQR] [C is involved in a disulfide bond]

- [1] Myers M., Richmond R.C., Oakeshott J.G. Mol. Biol. Evol. 5:113-119(1988).
- [2] Krejci E., Duval N., Chatonnet A., Vincens P., Massoulie J. Proc. Natl. Acad. Sci. U.S.A. 88:6647-6651(1991).
- [3] Cygler M., Schrag J.D., Sussman J.L., Harel M., Silman I. Gentry M.K., Doctor B.P. Protein Sci. 2:366-382(1993).

[4] Lockridge O. BioEssays 9:125-128(1988).

[5] Wang C.-S., Hartsuck J.A. Biochim. Biophys. Acta 1166:1-19(1993).

5 85. CPSase_L_chain (Carbamoyl-phosphate synthase (CPSase))

[1]

Medline: 94347758

Three-dimensional structure of the biotin carboxylase subunit.
of acetyl-CoA carboxylase.

10 Waldrop GL, Rayment I, Holden HM;

Biochemistry 1994;33:10249-10256.

[1]

Medline: 90285162

Mammalian carbamyl phosphate synthetase (CPS). DNA sequence and
evolution of the CPS domain of the Syrian hamster multifunctional
protein CAD.

15

Simmer JP, Kelly RE, Rinker AG Jr, Scully JL, Evans DR;

Biol Chem 1990;265:10395-10402.

Carbamoyl-phosphate synthase catalyzes the ATP-dependent synthesis of
carbamyl-phosphate from glutamine or ammonia and bicarbonate. This
important enzyme initiates both the urea cycle and the biosynthesis
of arginine and/or pyrimidines [2].

20

The carbamoyl-phosphate synthase (CPS) enzyme in prokaryotes is a
heterodimer of a small and large chain. The small chain promotes
the hydrolysis of glutamine to ammonia, which is used by the large
chain to synthesize carbamoyl phosphate. See CPSase_sm_chain.

25

The small chain has a GATase domain in the carboxyl terminus.

See GATase.

Number of members: 181

30

Carbamoyl-phosphate synthase (CPSase) catalyzes the ATP-dependent synthesis of
carbamyl-phosphate from glutamine (EC 6.3.5.5) or ammonia (EC 6.3.4.16) and
bicarbonate [1]. This important enzyme initiates both the urea cycle and the
biosynthesis of arginine and pyrimidines.

Glutamine-dependent CPSase (CPSase II) is involved in the biosynthesis of pyrimidines and purines. In bacteria such as *Escherichia coli*, a single enzyme is involved in both biosynthetic pathways while other bacteria have separate enzymes. The bacterial enzymes are formed of two subunits. A small chain (gene carA) that provides glutamine amidotransferase activity (GATase) necessary for removal of the ammonia group from glutamine, and a large chain (gene carB) that provides CPSase activity. Such a structure is also present in fungi for arginine biosynthesis (genes CPA1 and CPA2). In most eukaryotes, the first three steps of pyrimidine biosynthesis are catalyzed by a large multifunctional enzyme - called URA2 in yeast, rudimentary in *Drosophila* and CAD in mammals [2]. The CPSase domain is located between an N-terminal GATase domain and the C-terminal part which encompass the dihydroorotase and aspartate transcarbamylase activities.

Ammonia-dependent CPSase (CPSase I) is involved in the urea cycle in ureolytic vertebrates; it is a monofunctional protein located in the mitochondrial matrix.

The CPSase domain is typically 120 Kd in size and has arisen from the duplication of an ancestral subdomain of about 500 amino acids. Each subdomain independently binds to ATP and it is suggested that the two homologous halves act separately, one to catalyze the phosphorylation of bicarbonate to carboxy phosphate and the other that of carbamate to carbamyl phosphate.

The CPSase subdomain is also present in a single copy in the biotin-dependent enzymes acetyl-CoA carboxylase (EC 6.4.1.2) (ACC), propionyl-CoA carboxylase (EC 6.4.1.3) (PCCase), pyruvate carboxylase (EC 6.4.1.1) (PC) and urea carboxylase (EC 6.3.4.6).

Two conserved regions which are probably important for binding ATP and/or catalytic activity have been selected as signatures for the subdomain.

-Consensus pattern: [FYV]-[PS]-[LIVMC]-[LIVMA]-[LIVM]-[KR]-[PSA]-[STA]-x(3)-
[SG]-G-x-[AG]
-Consensus pattern: [LIVMF]-[LIMN]-E-[LIVMCA]-N-[PATLIVM]-[KR]-[LIVMSTAC]

- 5 [1] Simmer J.P., Kelly R.E., Rinker A.G. Jr., Scully J.L., Evans D.R.
J. Biol. Chem. 265:10395-10402(1990).
[2] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B.
BioEssays 15:157-164(1993).

10

86. CPSase_sm_chain (Carbamoyl-phosphate synthase small chain, CPSase domain)

[1]

Medline: 90285162

Mammalian carbamyl phosphate synthetase (CPS). DNA sequence and
evolution of the CPS domain of the Syrian hamster multifunctional
protein CAD.

Simmer JP, Kelly RE, Rinker AG Jr, Scully JL, Evans DR;
Biol Chem 1990;265:10395-10402.

The carbamoyl-phosphate synthase domain is in the amino terminus of
protein.

Carbamoyl-phosphate synthase catalyzes the ATP-dependent synthesis of
carbamyl-phosphate from glutamine or ammonia and bicarbonate. This
important enzyme initiates both the urea cycle and the biosynthesis
of arginine and/or pyrimidines [1].

The carbamoyl-phosphate synthase (CPS) enzyme in prokaryotes is a
heterodimer of a small and large chain. The small chain promotes
the hydrolysis of glutamine to ammonia, which is used by the large
chain to synthesize carbamoyl phosphate. See CPSase_L_chain.
The small chain has a GATase domain in the carboxyl terminus.

See GATase.

Number of members: 46

Carbamoyl-phosphate synthase (CPSase) catalyzes the ATP-dependent synthesis of
carbamyl-phosphate from glutamine (EC 6.3.5.5) or ammonia (EC 6.3.4.16) and

bicarbonate [1]. This important enzyme initiates both the urea cycle and the biosynthesis of arginine and pyrimidines.

Glutamine-dependent CPSase (CPSase II) is involved in the biosynthesis of pyrimidines and purines. In bacteria such as *Escherichia coli*, a single enzyme is involved in both biosynthetic pathways while other bacteria have separate enzymes. The bacterial enzymes are formed of two subunits. A small chain (gene *carA*) that provides glutamine amidotransferase activity (GATase) necessary for removal of the ammonia group from glutamine, and a large chain (gene *carB*) that provides CPSase activity. Such a structure is also present in fungi for arginine biosynthesis (genes *CPA1* and *CPA2*). In most eukaryotes, the first three steps of pyrimidine biosynthesis are catalyzed by a large multifunctional enzyme - called URA2 in yeast, rudimentary in *Drosophila* and CAD in mammals [2]. The CPSase domain is located between an N-terminal GATase domain and the C-terminal part which encompass the dihydroorotase and aspartate transcarbamylase activities.

Ammonia-dependent CPSase (CPSase I) is involved in the urea cycle in ureolytic vertebrates; it is a monofunctional protein located in the mitochondrial matrix.

The CPSase domain is typically 120 Kd in size and has arisen from the duplication of an ancestral subdomain of about 500 amino acids. Each subdomain independently binds to ATP and it is suggested that the two homologous halves act separately, one to catalyze the phosphorylation of bicarbonate to carboxy phosphate and the other that of carbamate to carbamyl phosphate.

The CPSase subdomain is also present in a single copy in the biotin-dependent enzymes acetyl-CoA carboxylase (EC 6.4.1.2) (ACC), propionyl-CoA carboxylase (EC 6.4.1.3) (PCCase), pyruvate carboxylase (EC 6.4.1.1) (PC) and urea carboxylase (EC 6.3.4.6).

Two conserved regions which are probably important for binding ATP and/or catalytic activity have been selected as signatures for the subdomain.

-Consensus pattern: [FYV]-[PS]-[LIVMC]-[LIVMA]-[LIVM]-[KR]-[PSA]-[STA]-x(3)-
[SG]-G-x-[AG]

-Consensus pattern: [LIVMF]-[LIMN]-E-[LIVMCA]-N-[PATLIVM]-[KR]-[LIVMSTAC]

5

[1] Simmer J.P., Kelly R.E., Rinker A.G. Jr., Scully J.L., Evans D.R.

J. Biol. Chem. 265:10395-10402(1990).

[2] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B.

BioEssays 15:157-164(1993).

10

87. CRAL_TRIO (CRAL/TRIO domain)

[1]

Medline: 98121119

15

Crystal structure of the *Saccharomyces cerevisiae* phosphatidyl-
inositol-transfer protein.

Sha B, Phillips SE, Bankaitis VA, Luo M;

Nature 1998;391:506-510.

The original profile has been extended to include the carboxyl

20

domain from the known structure of Sec14. Swiss:P10911 has not
been included in the Pfam family because it does not appear to
contain a complete structural domain.

Number of members: 39

25

88. CSD ('Cold-shock' DNA-binding domain)

[1]

Medline: 94255482

Crystal structure of CspA, the major cold shock

30

protein of *Escherichia coli*.

Schindelin H, Jiang W, Inouye M, Heinemann U;

Proc Natl Acad Sci U S A 1994;91:5119-5123.

Number of members: 121

A conserved domain of about 70 amino acids has been found in prokaryotic and eukaryotic DNA-binding proteins [1,2,3,E1]. This domain, which is known as the 'cold-shock domain' (CSD) is present in the proteins listed below.

- 5 - *Escherichia coli* protein CS7.4 (gene *cspA*) which is induced in response to low temperature (cold-shock protein) and which binds to and stimulates the transcription of the CCAAT-containing promoters of the *HN-S* protein and of *gyrA*.
- Mammalian Y box binding protein 1 (YB1). A protein that binds to the CCAAT-containing Y box of mammalian HLA class II genes.
- 10 - *Xenopus* Y box binding proteins -1 and -2 (Y1 and Y2). Proteins that bind to the CCAAT-containing Y box of *Xenopus hsp70* genes.
- *Xenopus* B box binding protein (YB3). YB3 binds the B box promoter element of genes transcribed by RNA polymerase III.
- 15 - Enhancer factor I subunit A (EFI-A) (*dbpB*). A protein that also bind to CCAAT-motif in various gene promoters.
- *DbpA*, a Human DNA-binding protein of unknown specificity.
- *Bacillus subtilis* cold-shock proteins *cspB* and *cspC*.
- *Streptomyces clavuligerus* protein SC 7.0.
- 20 - *Escherichia coli* proteins *cspB*, *cspC*, *cspD*, *cspE* and *cspF*.
- *Unr*, a mammalian gene encoded upstream of the *N-ras* gene. *Unr* contains nine repeats that are similar to the CSD domain. The function of *Unr* is not yet known but it could be a multivalent DNA-binding protein.
- 25 As a signature pattern for the CSD domain, its most conserved region which is located in its N-terminal section has been selected. It must be noted that the beginning of this region is highly similar [4] to the RNP-1 RNA-binding motif.

-Consensus pattern: [FY]-G-F-I-x(6,7)-[DER]-[LIVM]-F-x-H-x-[STKR]-x-[LIVMFY]

30

[1] Doniger J., Landsman D., Gonda M.A., Wistow G.

New Biol. 4:389-395(1992).

[2] Wistow G.

Nature 344:823-824(1990).

[3] Jones P.G., Inouye M.

Mol. Microbiol. 11:811-818(1994).

[4] Landsman D.

Nucleic Acids Res. 20:2861-2864(1992).

5

89. CTF_NFI (CTF/NF-I family)

Number of members: 45

10 Nuclear factor I (NF-I) or CCAAT box-binding transcription factor (CTF) [1,2]
(also known as TGGCA-binding proteins) are a family of vertebrate nuclear
proteins which recognize and bind, as dimers, the palindromic DNA sequence
5'-TGGCANNTGCCA-3'. CTF/NF-I binding sites are present in viral and cellular
promoters and in the origin of DNA replication of Adenovirus type 2.

15

The CTF/NF-I proteins were first identified as nuclear factor I, a collection
of proteins that activate the replication of several Adenovirus serotypes
(together with NF-II and NF-III) [3]. The family of proteins was also
identified as the CTF transcription factors, before the NFI and CTF families
20 were found to be identical [4]. The CTF/NF-I proteins are individually capable
of activating transcription and DNA replication. The CTF/NF-I family name has
also been dubbed as NFI, NF-I or NF1.

In a given species, there are a large number of different CTF/NF-I proteins.

25

The multiplicity of CTF/NF-I is known to be generated both by alternative
splicing and by the occurrence of four different genes. The known forms of
NF-I genes have been classified as:

30

- The CTF-like factors subfamily (prototype form: CTF-1) [4]
- The NFI-X proteins.
- The NFI-A proteins.
- The NFI-B proteins.

So far, all CTF/NF-I family members appear to have similar transcription and

replication activities.

CTF/NF-1 proteins contains 400 to 600 amino acids. The N-terminal 200 amino-acid sequence, almost perfectly conserved in all species and genes sequenced, mediates site-specific DNA recognition, protein dimerization and Adenovirus DNA replication. The C-terminal 100 amino acids contain the transcriptional activation domain. This activation domain is the target of gene expression regulatory pathways elicited by growth factors and it interacts with basal transcription factors and with histone H3 [6].

A perfectly conserved, highly charged 12 residue peptide located in the N-terminal part of CTF/NF-1 has been selected as a specific signature for this family of proteins.

-Consensus pattern: R-K-R-K-Y-F-K-K-H-E-K-R

[1] Mermod N., O'Neill E.A., Kelly T.J., Tjian R.

Cell 58:741-753(1989).

[2] Rupp R.A.W., Kruse U., Multhaup G., Goebel U., Beyreuther K.,

Sippel A.E.

Nucleic Acids Res. 18:2607-2616(1990).

[3] Nagata K., Guggenheimer R.A., Enomoto T., Lichy J.H., Hurwitz J.

Proc. Natl. Acad. Sci. U.S.A. 79:6438-6442(1982).

[4] Santoro C., Mermod N., Andrews P.C., Tjian R.

Nature 334:2118-2224(1988).

[5] Gil G., Smith J.R., Goldstein J.L., Slaughter C.A., Orth K., Brown M.S.,

Osborne T.F.

Proc. Natl. Acad. Sci. U.S.A 85:8963-8967(1988).

[6] Alevizopoulos A., Dusserre Y., Tsai-Pflugfelder M., von der Weid T.,

Wahli W., Mermod N.

Genes Dev. 9:3051-3066(1995).

90. Calsequestrin (Calsequestrin)

Number of members: 13

Calsequestrin is a moderate-affinity, high-capacity calcium-binding protein of cardiac and skeletal muscle [1], where it is located in the luminal space of the sarcoplasmic reticulum terminal cisternae. Calsequestrin acts as a calcium buffer and plays an important role in the muscle excitation-contraction coupling. It is a highly acidic protein of about 400 amino acid residues that binds more than 40 moles of calcium per mole of protein. There are at least two different forms of calsequestrin: one which is expressed in cardiac muscles and another in skeletal muscles. Both forms have highly similar sequences.

Two signature sequences have been developed. The first corresponds to the N-terminus of the mature protein, the second is located just in front of the C-terminus of the protein which is composed of a highly acidic tail of variable length.

-Consensus pattern: [EQ]-[DE]-G-L-[DN]-F-P-x-Y-D-G-x-D-R-V

-Consensus pattern: [DE]-L-E-D-W-[LIVM]-E-D-V-L-x-G-x-[LIVM]-N-T-E-D-D-D

[1] Treves S., Vilsen B., Chiozzi P., Andersen J.P., Zorzato F.
Biochem. J. 283:767-772(1992).

91. Carboxyl_trans (Carboxyl transferase domain)

[1]

Medline: 93374821

Primary structure of the monomer of the 12S subunit of transcarboxylase as deduced from DNA and characterization of the product expressed in Escherichia coli.

Thornton CG, Kumar GK, Haase FC, Phillips NF, Woo SB, Park VM, Magner WJ, Shenoy BC, Wood HG, Samols D;
J Bacteriol 1993;175:5301-5308.

[2]

Medline: 93358891

Molecular evolution of biotin-dependent carboxylases.

Toh H, Kondo H, Tanabe T;

Eur J Biochem 1993;215:687-696.

All of the members in this family are biotin dependent carboxylases.

- 5 The carboxyl transferase domain carries out the following reaction; transcarboxylation from biotin to an acceptor molecule. There are two recognised types of carboxyl transferase. One of them uses acyl-CoA and the other uses 2-oxo acid as the acceptor molecule of carbon dioxide. All of the members in this family utilise acyl-CoA as the acceptor molecule.

10 Number of members: 47

92. Chal_stil_synt (Chalcone and stilbene synthases)

- 15 Number of members: 146

Chalcone synthases (CHS) (EC 2.3.1.74) and stilbene synthases (STS) (formerly known as resveratrol synthases) are related plant enzymes [1]. CHS is an important enzyme in flavanoid biosynthesis and STS a key enzyme in stilbene-type phytoalexin biosynthesis. Both enzymes catalyze the addition of three molecules of malonyl-CoA to a starter CoA ester (a typical example is 4-coumaroyl-CoA), producing either a chalcone (with CHS) or stilbene (with STS).

- 25 These enzymes are proteins of about 390 amino-acid residues. A conserved cysteine residue, located in the central section of these proteins, has been shown [2] to be essential for the catalytic activity of both enzymes and probably represents the binding site for the 4-coumaroyl-CoA group. The region around this active site residue is well conserved and can be used as a signature pattern.

In addition to the plant enzymes, this family also includes *Bacillus subtilis* bcsA.

-Consensus pattern: R-[LIVMFYS]-x-[LIVM]-x-[QHG]-x-G-C-[FYNA]-[GA]-G-[GA]-[STAV]-x-[LIVMF]-[RA] [C is the active site residue]

[1] Schroeder J., Schroeder G.

5 Z. Naturforsch. 45C:1-8(1990).

[2] Lanz T., Tropf S., Marner F.-J., Schroeder J., Schroeder G.

J. Biol. Chem. 266:9971-9976(1991).

10 93. Chorismate_synt (Chorismate synthase)

Number of members: 19

Chorismate synthase (EC 4.6.1.4) catalyzes the last of the seven steps in the shikimate pathway which is used in prokaryotes, fungi and plants for the biosynthesis of aromatic amino acids. It catalyzes the 1,4-trans elimination of the phosphate group from 5-enolpyruvylshikimate-3-phosphate (EPSP) to form chorismate which can then be used in phenylalanine, tyrosine or tryptophan biosynthesis. Chorismate synthase requires the presence of a reduced flavin mononucleotide (FMNH2 or FADH2) for its activity.

20

Chorismate synthase from various sources shows [1,2] a high degree of sequence conservation. It is a protein of about 360 to 400 amino-acid residues. Three signature patterns have been developed from conserved regions rich in basic residues (mostly arginines). The first is in the N-terminal section, the second is central and the third is C-terminal.

25

-Consensus pattern: G-E-S-H-[GC]-x(2)-[LIVM]-[GTV]-x-[LIVM](2)-[DE]-G-x-[PV]

-Consensus pattern: [GE]-R-[SA](2)-[SAG]-R-[EV]-[ST]-x(2)-[RH]-V-x(2)-G

30

-Consensus pattern: R-[SH]-D-[PSV]-[CSAV]-x(4)-[GAI]-x-[IVGSP]-[LIVM]-x-E-[STAH]-[LIVM]

[1] Schaller A., Schmid J., Leibinger U., Amrhein N.

J. Biol. Chem. 266:21434-21438(1991).

[2] Jones D.G.L., Reusser U., Braus G.H.

Mol. Microbiol. 5:2143-2152(1991).

5 94. Clat_adaptor_s (Clathrin adaptor complex small chain)

Number of members: 21

Clathrin coated vesicles (CCV) mediate intracellular membrane traffic such as receptor mediated endocytosis. In addition to clathrin, the CCV are composed of a number of other components including oligomeric complexes which are known as adaptor or clathrin assembly proteins (AP) complexes [1]. The adaptor complexes are believed to interact with the cytoplasmic tails of membrane proteins, leading to their selection and concentration. In mammals two type of adaptor complexes are known: AP-1 which is associated with the Golgi complex and AP-2 which is associated with the plasma membrane. Both AP-1 and AP-2 are heterotetramers that consist of two large chains - the adaptins - (gamma and beta' in AP-1; alpha and beta in AP-2); a medium chain (AP47 in AP-1; AP50 in AP-2) and a small chain (AP19 in AP-1; AP17 in AP-2).

The small chains of AP-1 and AP-2 are evolutionary related proteins of about 18 Kd. Homologs of AP17 and AP19 have also been found in yeast (genes APS1/YAP19 and APS2/YAP17) [2,3,4]. AP17 and AP19 are also related to the zeta-chain [5] of coatmer (zeta-cop), a cytosolic protein complex that reversibly associates with Golgi membranes to form vesicles that mediate biosynthetic protein transport from the endoplasmic reticulum, via the Golgi up to the trans Golgi network.

A conserved region in the central section of these proteins has been selected as a signature pattern.

-Consensus pattern: [LIVM](2)-Y-[KR]-x(4)-L-Y-F

[1] Pearse B.M., Robinson M.S.

Annu. Rev. Cell Biol. 6:151-171(1990).

- [2] Kirchhausen T., Davis A.C., Frucht S., O'Brine Greco B., Payne G.S.,
Tubb B.
J. Biol. Chem. 266:11153-11157(1991).
- [3] Nakai M., Takada T., Endo T.
5 Biochim. Biophys. Acta 1174:282-284(1993).
- [4] Phan H.L., Finlay J.A., Chu D.S., Tan P.K., Kirchhausen T., Payne G.S.
EMBO J. 13:1706-1717(1994).
- [5] Kuge O., Hara-Kuge S., Orci L., Ravazzola M., Amherdt M., Tanigawa G.,
Wieland F.T., Rothman J.E.
10 J. Cell Biol. 123:1727-1734(1993).

95. Clathrin_lg_ch (Clathrin light chain.)

Number of members: 8

15 Clathrin [1,2] is the major coat-forming protein that encloses vesicles such as coated pits and forms cell surface patches involved in membrane traffic within eukaryotic cells. The clathrin coats (called triskelions) are composed of three heavy chains (180 Kd) and three light chains (23 to 27 Kd).

20 The clathrin light chains [3], which may help to properly orient the assembly and disassembly of the clathrin coats, bind non-covalently to the heavy chain, they also bind calcium and interact with the hsc70 uncoating ATPase.

- 25 - In higher eukaryotes two genes code for distinct but related light chains: LC(a) and LC(b). Each of the two genes can yield, by tissue-specific alternative splicing, two separate forms which differ by the insertion of a sequence of respectively thirty or eighteen residues. There is, in the N-terminal part of the clathrin light chains a domain of twenty one amino
30 acid residues which is perfectly conserved in LC(a) and LC(b).
- In yeast there is a single light chain (gene CLC1) whose sequence is only distantly related to that of higher eukaryotes.

Two signature patterns have been developed for clathrin light chains. The first

pattern is a heptapeptide from the center of the conserved N-terminal region of eukaryotic light chains; the second pattern is derived from a positively charged region located in the C-terminal extremity of all known clathrin light chains.

5

-Consensus pattern: F-L-A-Q-Q-E-S

[1] Keen J.H.

Annu. Rev. Biochem. 59:415-438(1990).

10

[2] Brodsky F.M.

Science 242:1396-1402(1988).

[3] Brodsky F.M., Hill B.L., Acton S.L., Naethke I., Wong D.H.,
Ponnambalam S., Parham P.

Trends Biochem. Sci. 16:208-213(1991).

15

96. (Clathrin repeat) 7-fold repeat in Clathrin and VPS

Each repeat is about 140 amino acids long. The repeats
occur in the arm region of the Clathrin heavy chain.

20

Number of members: 79

[1]

Medline: 92191269

Folding and trimerization of clathrin subunits at the
triskelion hub.

25

Nathke IS, Heuser J, Lupas A, Stock J, Turck CW, Brodsky FM;

Cell 1992;68:899-910. [2]

Medline: 88097376

Clathrin heavy chain: molecular cloning and complete primary
structure.

30

Kirchhausen T, Harrison SC, Chow EP, Mattaliano RJ,

Ramachandran KL, Smart J, Brosius J;

Proc Natl Acad Sci U S A 1987;84:8805-8809.

97. Collagen (Collagen triple helix repeat (20 copies))

[1] Medline: 94059583

New members of the collagen superfamily

Mayne R, Brewton RG;

5 Curr Opin Cell Biol 1993;5:883-890.

Scurvy is associated with collagens.

Members of this family belong to the collagen superfamily [1].

Collagens are generally extracellular structural proteins involved in formation of connective tissue structure.

10 The alignment contains 20 copies of the G-X-Y repeat that

forms a triple helix. The first position of the repeat is glycine, the second and third positions can be any residue but are frequently proline and hydroxyproline. Collagens are post translationally modified by proline hydroxylase

15 to form the hydroxyproline residues. Defective

hydroxylation is the cause of scurvy.

Some members of the collagen superfamily are not involved in connective tissue structure but share the same triple helical structure.

20 Number of members: 2125

98. Coprogen_oxidas (Coproporphyrinogen III oxidase)

Number of members: 12

25 Coproporphyrinogen III oxidase (EC 1.3.3.3) (coproporphyrinogenase) [1,2] catalyzes the oxidative decarboxylation of coproporphyrinogen III into protoporphyrinogen IX, a common step in the pathway for the biosynthesis of porphyrins such as heme, chlorophyll or cobalamin.

30 Coproporphyrinogen III oxidase is an enzyme that requires iron for its activity. A cysteine seems to be important for the catalytic mechanism [3]. Sequences from a variety of eukaryotic and prokaryotic sources show that this enzyme has been evolutionarily conserved. A highly conserved region in the central part of the sequence has been selected as a signature pattern. This

region contains the only conserved cysteine and is rich in charged amino acids.

-Consensus pattern: K-x-W-C-x(2)-[FYH](3)-[LIVM]-x-H-R-x-E-x-R-G-[LIVM]-G-G-
5 [LIVM]-F-F-D

[1] Xu K., Elliott T.

J. Bacteriol. 175:4990-4999(1993).

[2] Kohno H., Furukawa T., Yoshinaga T., Tokunaga R., Taketani S.

J. Biol. Chem. 268:21359-21363(1993).

[3] Camadro J.M., Chambon H., Jolles J., Labbe P.

Eur. J. Biochem. 156:579-587(1986).

[4] Xu K., Elliott T.

J. Bacteriol. 176:3196-3203(1994).

99. Corona_nucleoca (Coronavirus nucleocapsid protein)

[1]

Medline: 98087828

20 Identification of a specific interaction between the
coronavirus mouse hepatitis virus A59 nucleocapsid protein
and packaging signal.

Molenkamp R, Spaan WJ;

Virology 1997;239:78-86.

25 Number of members: 44

100. Cu-oxidase (Multicopper oxidase)

[1]

30 Medline: 90126844

The blue oxidases, ascorbate oxidase, laccase and ceruloplasmin.

Modelling and structural relationships.

Messerschmidt A, Huber R;

Eur J Biochem 1990;187:341-352.

Number of members: 150

Multicopper oxidases [1,2] are enzymes that possess three spectroscopically different copper centers. These centers are called: type 1 (or blue), type 2
5 (or normal) and type 3 (or coupled binuclear). The enzymes that belong to this family are:

- Laccase (EC 1.10.3.2) (urishiol oxidase), an enzyme found in fungi and plants, which oxidizes many different types of phenols and diamines.
- 10 - Ascorbate oxidase (EC 1.10.3.3), a higher plant enzyme.
- Ceruloplasmin (EC 1.16.3.1) (ferroxidase), a protein found in the serum of mammals and birds, which oxidizes a great variety of inorganic and organic substances. Structurally ceruloplasmin exhibits internal sequence homology, and seem to have evolved from the triplication of a copper-binding domain
15 similar to that found in laccase and ascorbate oxidase.

In addition to the above enzymes there are a number of proteins which, on the basis of sequence similarities, can be said to belong to this family. These proteins are:

- 20 - Copper resistance protein A (copA) from a plasmid in *Pseudomonas syringae*. This protein seems to be involved in the resistance of the microbial host to copper.
- Blood coagulation factor V (Fa V).
- 25 - Blood coagulation factor VIII (Fa VIII) [E1].
- Yeast FET3 [3], which is required for ferrous iron uptake.
- Yeast hypothetical protein YFL041w and SpAC1F7.08, the fission yeast homolog.

30 Factors V and VIII act as cofactors in blood coagulation and are structurally similar [4]. Their sequence consists of a triplicated A domain, a B domain and a duplicated C domain; in the following order: A-A-B-A-C-C. The A-type domain is related to the multicopper oxidases.

Two signature patterns have been developed for these proteins. Both patterns are derived from the same region, which in ascorbate oxidase, laccase, in the third domain of ceruloplasmin, and in copA, contains five residues that are known to be involved in the binding of copper centers. The first pattern does not make any assumption on the presence of copper-binding residues and thus can detect domains that have lost the ability to bind copper (such as those in Fa V and Fa VIII), while the second pattern is specific to copper-binding domains.

-Consensus pattern: G-x-[FYW]-x-[LIVMFYW]-x-[CST]-x(8)-G-[LM]-x(3)-[LIVMFYW]

-Consensus pattern: H-C-H-x(3)-H-x(3)-[AG]-[LM]

[The first two H's are copper type 3 binding residues]

[The C, the 3rd H, and L or M are copper type 1 ligands]

101. Cullin (Cullin family)

Number of members: 24

The following proteins are collectively termed cullins [1]:

- *Caenorhabditis elegans* cul-1 (or lin-19), a protein required for developmentally programmed transitions from the G1 phase of the cell cycle to the G0 phase or the apoptotic pathway.
- *Caenorhabditis elegans* cul-2, cul-3, cul-4 (F45E12.3), cul-5 (ZK856.1) and cul-6 (K08E7.7).
- Mammalian CUL1, CUL2, CUL3, CUL4A and CUL4B.
- Mammalian vasopressin-activated calcium-mobilizing receptor (VACM-1), a kidney-specific protein thought to form a cell surface receptor [2] but which does not have any structural hallmarks of a receptor.
- *Drosophila* lin19.
- Yeast CDC53 [3], which acts in concert with CDC4 and UBC3 (CDC34) to control the G1-to-S phase transition.
- Yeast hypothetical protein YGR003w.
- Fission yeast hypothetical protein SpAC24H6.03.

The cullins are hydrophilic proteins of 740 to 815 amino acids. The C-terminal extremity is the most conserved part of these proteins. A signature pattern has been developed from that region.

-Consensus pattern: [LIV]-K-x(2)-[LIV]-x(2)-L-I-[DEQ]-[KRHNQ]-x-Y-[LIVM]-x-R-x(6,7)-[FY]-x-Y-x-[SA]>

[1] Kipreos E.T., Lander L.E., Wing J.P., He W.W., Hedgecock E.M.

Cell 85:829-839(1996).

[2] Burnatowska-Hledin M.A., Spielman W.S., Smith W.L., Shi P., Meyer J.M., Dewitt D.L.

Am. J. Physiol. 268:f1198-F1210(1995).

[3] Mathias N., Johnson S.L., Winey M., Adams A.E., Goetsch L., Pringle J.R.,

Byers B., Goebel M.G.

Mol. Cell. Biol. 16:6634-6643(1996).

102. (Cu_amine_oxid)

Copper amine oxidase signatures

Amine oxidases (AO) [1] are enzymes that catalyze the oxidation of a wide range of biogenic amines including many neurotransmitters, histamine and xenobiotic amines. There are two classes of amine oxidases: flavin-containing (EC 1.4.3.4) and copper-containing (EC 1.4.3.6).

Copper-containing AO is found in bacteria, fungi, plants and animals, it is an homodimeric enzyme that binds one copper ion per subunit as well as a 2,4,5- trihydroxyphenylalanine quinone (or topaquinone) (TPQ) cofactor. This cofactor is derived from a tyrosine residue.

Two signature patterns were derived for copper AO, the first one contains the tyrosine which give rises to the TPQ cofactor while the second one contains one of the three histidines that bind the copper atom [2].

Consensus pattern[LIVM]-[LIVMA]-[LIVMF]-x(4)-[ST]-x(2)-N-Y-[DE]-[YN] [The first Y gives rises to TPQ] Sequences known to belong to this class detected by the patternALL.

Consensus pattern T-x-[GS]-x(2)-H-[LIVMF]-x(3)-E-[DE]-x-P [H is a copper ligand]
Sequences known to belong to this class detected by the pattern ALL, except for lentil AO.

- 5 [1] Knowles P.F., Dooley D.M. (In) Metal ions in biological systems; Sigel H., Sigel A., Eds., 30:361- 403, Marcel Dekker, New-York, (1993).
- [2] Parsons M.R., Convery M.A., Wilmot C.M., Yadav K.D.S., Blakeley V., Corner A.S., Phillips S.E.V., McPherson M.J., Knowles P.F. Structure 3:1171-1184(1995).

10

103. Cys-protease (Cysteine protease)

Number of members: 358

Eukaryotic thiol proteases (EC 3.4.22.-) [1] are a family of proteolytic enzymes which contain an active site cysteine. Catalysis proceeds through a thioester intermediate and is facilitated by a nearby histidine side chain; an asparagine completes the essential catalytic triad. The proteases which are currently known to belong to this family are listed below (references are only provided for recently determined sequences).

20

- Vertebrate lysosomal cathepsins B (EC 3.4.22.1), H (EC 3.4.22.16), L (EC 3.4.22.15), and S (EC 3.4.22.27) [2].
- Vertebrate lysosomal dipeptidyl peptidase I (EC 3.4.14.1) (also known as cathepsin C) [2].
- 25 - Vertebrate calpains (EC 3.4.22.17). Calpains are intracellular calcium-activated thiol protease that contain both a N-terminal catalytic domain and a C-terminal calcium-binding domain.
- Mammalian cathepsin K, which seems involved in osteoclastic bone resorption [3].
- 30 - Human cathepsin O [4].
- Bleomycin hydrolase. An enzyme that catalyzes the inactivation of the antitumor drug BLM (a glycopeptide).
- Plant enzymes: barley aleurain (EC 3.4.22.16), EP-B1/B4; kidney bean EP-C1, rice bean SH-EP; kiwi fruit actinidin (EC 3.4.22.14); papaya latex papain

(EC 3.4.22.2), chymopapain (EC 3.4.22.6), caricain (EC 3.4.22.30), and proteinase IV (EC 3.4.22.25); pea turgor-responsive protein 15A; pineapple stem bromelain (EC 3.4.22.32); rape COT44; rice oryzain alpha, beta, and gamma; tomato low-temperature induced, *Arabidopsis thaliana* A494, RD19A and

RD21A.

- House-dust mites allergens DerP1 and EurM1.

- Cathepsin B-like proteinases from the worms *Caenorhabditis elegans* (genes gcp-1, cpr-3, cpr-4, cpr-5 and cpr-6), *Schistosoma mansoni* (antigen SM31) and *Japonica* (antigen SJ31), *Haemonchus contortus* (genes AC-1 and AC-2), and *Ostertagia ostertagi* (CP-1 and CP-3).

- Slime mold cysteine proteinases CP1 and CP2.

- Cruzipain from *Trypanosoma cruzi* and *brucei*.

- Throphozoite cysteine proteinase (TCP) from various *Plasmodium* species.

- Proteases from *Leishmania mexicana*, *Theileria annulata* and *Theileria parva*.

- Baculoviruses cathepsin-like enzyme (v-cath).

- *Drosophila* small optic lobes protein (gene sol), a neuronal protein that contains a calpain-like domain.

- Yeast thiol protease BLH1/YCP1/LAP3.

- *Caenorhabditis elegans* hypothetical protein C06G4.2, a calpain-like protein.

Two bacterial peptidases are also part of this family:

- Aminopeptidase C from *Lactococcus lactis* (gene pepC) [5].

- Thiol protease tpr from *Porphyromonas gingivalis*.

Three other proteins are structurally related to this family, but may have lost their proteolytic activity.

- Soybean oil body protein P34. This protein has its active site cysteine replaced by a glycine.

- Rat testin, a sertoli cell secretory protein highly similar to cathepsin L but with the active site cysteine is replaced by a serine. Rat testin should not be confused with mouse testin which is a LIM-domain protein (see

<PDOC00382>).

- Plasmodium falciparum serine-repeat protein (SERA), the major blood stage antigen. This protein of 111 Kd possesses a C-terminal thiol-protease-like domain [6], but the active site cysteine is replaced by a serine.

5

The sequences around the three active site residues are well conserved and can be used as signature patterns.

-Consensus pattern: Q-x(3)-[GE]-x-C-[YW]-x(2)-[STAGC]-[STAGCV] [C is the active site residue]

10

-Consensus pattern: [LIVMGSTAN]-x-H-[GSACE]-[LIVM]-x-[LIVMAT](2)-G-x-[GSADNH] [H is the active site residue]

-Consensus pattern: [FYCH]-[WI]-[LIVT]-x-[KROAG]-N-[ST]-W-x(3)-[FYW]-G-x(2)-G-[LFYW]-[LIVMFY]-x-[LIVMF] [N is the active site residue]

15

[1] Dufour E. Biochimie 70:1335-1342(1988).

[2] Kirschke H., Barrett A.J., Rawlings N.D. Protein Prof. 2:1587-1643(1995).

[3] Shi G.-P., Chapman H.A., Bhairi S.M., Deleuw C., Reddy V.Y., Weiss S.J. FEBS Lett. 357:129-134(1995).

20

[4] Velasco G., Ferrando A.A., Puente X.S., Sanchez L.M., Lopez-Otin C. J. Biol. Chem. 269:27136-27142(1994).

[5] Chapot-Chartier M.P., Nardi M., Chopin M.C., Chopin A., Gripon J.C. Appl. Environ. Microbiol. 59:330-333(1993).

25

[6] Higgins D.G., McConnell D.J., Sharp P.M. Nature 340:604-604(1989).

[7] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:461-486(1994).

104. Cys_Met_Meta_PP (Cys/Met metabolism PLP-dependent enzyme)

30

[1] Medline: 96428687

Crystal structure of the pyridoxal-5'-phosphate dependent cystathionine beta-lyase from Escherichia coli at 1.83 Å.

Clausen T, Huber R, Laber B, Pohlenz HD, Messerschmidt A;

J Mol Biol 1996;262:202-224.

[1] Medline: 99059720

Crystal structure of Escherichia coli cystathionine
gamma-synthase at 1.5 Å resolution.

Clausen T, Huber R, Prade L, Wahl MC, Messerschmidt A;

5 EMBO J 1998;17:6827-6838.

Database Reference: SCOP; 1cs1; fa; [SCOP-USA][CATH-PDBSUM]

This family includes enzymes involved in cysteine and
methionine metabolism. The following are members:

Cystathionine gamma-lyase,

10 Cystathionine gamma-synthase,

Cystathionine beta-lyase,

Methionine gamma-lyase,

OAH/OAS sulfhydrylase,

O-succinylhomoserine sulphhydrylase

15 All of these members participate in slightly different reactions.

All these enzymes use PLP (pyridoxal-5'-phosphate) as a cofactor.

Number of members: 52

A number of pyridoxal-dependent enzymes involved in the metabolism of
20 cysteine, homocysteine and methionine have been shown [1,2] to be evolutionary
related. These are:

- Cystathionine gamma-lyase (EC 4.4.1.1) (gamma-cystathionase), which
catalyzes the transformation of cystathionine into cysteine, oxobutanoate
25 and ammonia. This is the final reaction in the transsulfuration pathway that
leads from methionine to cysteine in eukaryotes.

- Cystathionine gamma-synthase (EC 4.2.99.9), which catalyzes the conversion
of cysteine and succinyl-homoserine into cystathionine and succinate: the
first step in the biosynthesis of methionine from cysteine in bacteria
30 (gene metB).

- Cystathionine beta-lyase (EC 4.4.1.8) (beta-cystathionase), which catalyzes
the conversion of cystathionine into homocysteine, pyruvate and ammonia:
the second step in the biosynthesis of methionine from cysteine in bacteria
(gene metC).

- Methionine gamma-lyase (EC 4.4.1.11) (L-methioninase) which catalyzes the transformation of methionine into methanethiol, oxobutanoate and ammonia.
- OAH/OAS sulfhydrylase, which catalyzes the conversion of acetylhomoserine into homocysteine and that of acetylserine into cysteine (gene MET17 or MET25 in yeast).
- O-succinylhomoserine sulfhydrylase (EC 4.2.99.-).
- Yeast hypothetical protein YGL184c.
- Yeast hypothetical protein YHR112c.

These enzymes are proteins of about 400 amino-acid residues. The pyridoxal-P group is attached to a lysine residue located in the central section of these enzymes; the sequence around this residue is highly conserved and can be used as a signature pattern to detect this class of enzymes.

-Consensus pattern: [DQ]-[LIVMF]-x(3)-[STAGC]-[STAGCI]-T-K-[FYWQ]-[LIVMF]-x-G-[HO]-[SGNH] [K is the pyridoxal-P attachment site]

[1] Ono B.I., Tanaka K., Naito K., Heike C., Shinoda S., Yamamoto S., Ohmori S., Oshima T., Toh-E A.

J. Bacteriol. 174:3339-3347(1992).

[2] Barton A.B., Kaback D.B., Clark M.W., Keng T., Ouellette B.F.F., Storms R.K., Zeng B., Zhong W.W., Fortin N., Delaney S., Bussey H. Yeast 9:363-369(1993).

105. Cyt_r reductase

FAD/NAD-binding Cytochrome reductase

Number of members: 60

[1] Medline: 95111952

Crystal structure of the FAD-containing fragment of corn nitrate reductase at 2.5 Å resolution: relationship to other flavoprotein reductases.

Lu G, Campbell WH, Schneider G, Lindqvist Y;

Structure 1994;2:809-821.

[2] Medline: 92084635

The sequence of squash NADH:nitrate reductase and its relationship to the sequences of other flavoprotein oxidoreductases. A family of flavoprotein pyridine

5 nucleotide cytochrome reductases.

Hyde GE, Crawford NM, Campbell WH;

J Biol Chem 1991;266:23542-23547.

10 106. Cytidyltrans

Phosphatidate cytidyltransferase

Number of members: 21

Phosphatidate cytidyltransferase (EC 2.7.7.41) [1,2,3] (also known as CDP-

15 diacylglycerol synthase) (CDS) is the enzyme that catalyzes the synthesis of CDP-diacylglycerol from CTP and phosphatidate (PA). CDP-diacylglycerol is an important branch point intermediate in both prokaryotic and eukaryotic organisms. CDS is a membrane-bound enzyme. A conserved region located in the C-terminal part has been selected as a signature pattern.

20

-Consensus pattern: S-x-[LIVMF]-K-R-x(4)-K-D-x-[GSA]-x(2)-[LI]-[PG]-x-H-G-G-[LIVM]-x-D-R-[LIVMF]-D

[1] Sparrow C.P., Raetz C.R.H.

25 J. Biol. Chem. 260:12084-12091(1985).

[2] Shen H., Heacock P.N., Clancey C.J., Dowhan W.

J. Biol. Chem. 271:789-795(1996).

[3] Saito S., Goto K., Tonosaki A., Kondo H.

J. Biol. Chem. 272:9503-9509(1997).

30

107. (Cytidyltransf) Cytidyltransferase. This family includes: Cholinephosphate cytidyltransferase. Glycerol-3-phosphate cytidyltransferase.

Number of members: 64

[1] Medline: 10208837 CTP:Phosphocholine Cytidyltransferase: Insights into Regulatory Mechanisms and Novel Functions. Clement JM, Kent C; Biochem Biophys Res Commun 1999;257:643-650.

5

108. (cNMP binding) Cyclic nucleotide-binding domain signatures and profile
 Proteins that bind cyclic nucleotides (cAMP or cGMP) share a structural domain of about 120
 residues [1-3]. The best studied of these proteins is the prokaryotic catabolite gene activator
 10 (also known as the cAMP receptor protein) (gene *crp*) where such a domain is known to be
 composed of three α -helices and a distinctive eight-stranded, antiparallel β -
 barrel structure. Such a domain is known to exist in the following proteins: - Prokaryotic
 catabolite gene activator protein (CAP). - cAMP- and cGMP-dependent protein kinases
 (cAPK and cGPK). Both types of kinases contain two tandem copies of the cyclic
 15 nucleotide-binding domain. The cAPK's are composed of two different subunits: a catalytic
 chain and a regulatory chain which contains both copies of the domain. The cGPK's are
 single chain enzymes that include the two copies of the domain in their N- terminal section.
 The nucleotide specificity of cAPK and cGPK is due to an amino acid in the conserved
 region of β -barrel 7: a threonine that is invariant in cGPK is an alanine in most cAPK. -
 20 Vertebrate cyclic nucleotide-gated ion-channels. Two such cation channels have been fully
 characterized. One is found in rod cells where it plays a role in visual signal transduction. It
 specifically binds to cGMP leading to an opening of the channel and thereby causing a
 depolarization of rod photoreceptors. In olfactory epithelium a similar, cAMP-binding,
 channel plays a role in odorant signal transduction. There are six invariant amino acids in
 25 this domain, three of which are glycine residues that are thought to be essential for
 maintenance of the β -barrel. Two signature patterns for this domain have been
 developed. The first pattern is located within β -barrels 2 and 3 and contains the first two
 conserved Gly. The second pattern is located within β -barrels 6 and 7 and contains the
 third conserved Gly as well as the three other invariant residues.-
 30 First consensus pattern: [LIVM]-[VIC]-x(2)-G-[DENQTA]-x-[GAC]-x(2)-[LIVMFY](4)-
 x(2)-G
 Second consensus pattern: [LIVMF]-G-E-x-[GAS]-[LIVM]-x(5,11)-R-[STAQ]-A-x-
 [LIVMA]-x-[STACV]-

[1] Weber I.T., Shabb J.B., Corbin J.D. Biochemistry 28:6122-6127(1989).

[2] Kaupp U.B. Trends Neurosci. 14:150-157(1991).

[3] Shabb J.B., Corbin J.D. J. Biol. Chem. 267:5723-5726(1992).

5

109. (cadherin)

Cadherins extracellular repeated domain signature

Cadherins [1,2] are a family of animal glycoproteins responsible for calcium-dependent cell-cell adhesion. Cadherins preferentially interact with themselves in a homophilic manner in connecting cells; thus acting as both receptor and ligand. A wide number of tissue-specific forms of cadherins are known:

10

- Epithelial (E-cadherin) (also known as uvomorulin or L-CAM) (CDH1).

- Neural (N-cadherin) (CDH2).

15

- Placental (P-cadherin) (CDH3).

- Retinal (R-cadherin) (CDH4).

- Vascular endothelial (VE-cadherin) (CDH5).

- Kidney (K-cadherin) (CDH6).

- Cadherin-8 (CDH8).

20

- Osteoblast (OB-cadherin) (CDH11).

- Brain (BR-cadherin) (CDH12).

- T-cadherin (truncated cadherin) (CDH13).

- Muscle (M-cadherin) (CDH14).

- Liver-intestine (LI-cadherin).

25

- EP-cadherin.

Structurally, cadherins are built of the following domains: a signal sequence, followed by a propeptide of about 130 residues, then an extracellular domain of around 600 residues, then a transmembrane region, and finally a C-terminal cytoplasmic domain of about 150 residues.

30

The extracellular domain can be sub- divided into five parts: there are four repeats of about 110 residues followed by a region that contains four conserved cysteines. It is suggested that the calcium-binding region of cadherins is located in the extracellular repeats.

Cadherins are evolutionary related to the desmogleins which are component of intercellular desmosome junctions involved in the interaction of plaque proteins:

- Desmoglein 1 (desmosomal glycoprotein I).
- 5 - Desmoglein 2.
- Desmoglein 3 (Pemphigus vulgaris antigen).

The Drosophila fat protein [3] is a huge protein of over 5000 amino acids that contains 34 cadherin-like repeats in its extracellular domain.

10 The signature pattern that was developed for the repeated domain is located in it the C-terminal extremity which is its best conserved region. The pattern includes two conserved aspartic acid residues as well as two asparagines; these residues could be implicated in the binding of calcium.

15 Consensus pattern[LIV]-x-[LIV]-x-D-x-N-D-[NH]-x-P Sequences known to belong to this class detected by the pattern ALL. Note this pattern is found in the first, second, and fourth copies of the repeated domain. In the third copy there is a deletion of one residue after the second conserved Asp.

20 [1] Takeichi M. Annu. Rev. Biochem. 59:237-252(1990).

[2] Takeichi M. Trends Genet. 3:213-217(1987).

[3] Mahoney P.A., Weber U., Onofrechuk P., Biessmann H., Bryant P.J., Goodman C.S. Cell 67:853-868(1991).

25 110. Calreticulin family signatures

Calreticulin [1] (also known as calregulin, CRP55 or HACBP) is a high-capacitycalcium-binding protein which is present in most tissues and located at the periphery of the
30 endoplasmic (ER) and the sarcoplasmic reticulum (SR)membranes. It probably plays a role in the storage of calcium in the lumen ofthe ER and SR and it may well have other important functions. Structurally, calreticulin is a protein of about 400 amino acid residues consisting of three domains: a) An N-terminal, probably globular, domain of about 180 amino acid residues (N-domain); b) A central domain of about 70 residues (P-domain) which contains

three repeats of an acidic 17 amino acid motif. This region binds calcium with a low-capacity, but a high-affinity; c) A C-terminal domain rich in acidic residues and in lysine (C-domain). This region binds calcium with a high-capacity but a low-affinity. Calreticulin is evolutionary related to the following proteins: - Onchocerca volvulus antigen RAL-1. RAL-1 is highly similar to calreticulin, but possesses a C-terminal domain rich in lysine and arginine and lacks acidic residues and is therefore not expected to bind calcium in that region. - Calnexin [2]. A calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It seems to play a major role in the quality control apparatus of the ER by the retention of incorrectly folded proteins. - Calmegin [3] (or calnexin-T), a testis-specific calcium-binding protein highly similar to calnexin. Three signature patterns have been developed for this family of proteins. The first two patterns are based on conserved regions in the N-domain; the third pattern corresponds to positions 4 to 16 of the repeated motif in the P-domain.

Consensus pattern: [KRHN]-x-[DEQN]-[DEQNK]-x(3)-C-G-G-[AG]-[FY]-[LIVM]-[KN]-[LIVMFY](2)-

Consensus pattern: [LIVM](2)-F-G-P-D-x-C-[AG]-

Consensus pattern: [IV]-x-D-x-[DENST]-x(2)-K-P-[DEH]-D-W-[DEN]-

[1] Michalak M., Milner R.E., Burns K., Opas M. Biochem. J. 285:681-692(1992).

[2] Bergeron J.J.M., Brenner M.B., Thomas D.Y., Williams D.B. Trends Biochem. Sci. 19:124-128(1994).

[3] Watanabe D., Yamada K., Nishina Y., Tajima Y., Koshimizu U., Nagata A., Nishimune Y. J. Biol. Chem. 269:7744-7749(1994).

111. Eukaryotic-type carbonic anhydrases signature (carb_anhydrase)

Carbonic anhydrases (EC 4.2.1.1) (CA) [1,2,3,4] are zinc metalloenzymes which catalyze the reversible hydration of carbon dioxide. Eight enzymatic and evolutionary related forms of carbonic anhydrase are currently known to exist in vertebrates: three cytosolic isozymes (CA-I, CA-II and CA-III); two membrane-bound forms (CA-IV and CA-VII); a mitochondrial form (CA-V); a secreted salivary form (CA-VI); and a yet uncharacterized isozyme [5]. In the alga *Chlamydomonas reinhardtii*, two CA isozymes have been sequenced [6]. They are periplasmic glycoproteins evolutionary related to vertebrate CAs. Some bacteria, such as *Neisseria gonorrhoeae* [7] also have a eukaryotic-type CA. CAs contain a single zinc atom

bound to three conserved histidine residues. As a signature for CAs, a pattern has been developed which includes one of these zinc-binding histidines. Protein D8 from Vaccinia and other poxviruses is related to CAs but has lost two of the zinc-binding histidines as well as many otherwise conserved residues. This is also true of the N-terminal extracellular domain of some receptor-type tyrosine-protein phosphatases (see <PDOC00323>).

Consensus pattern: S-E-[HN]-x-[LIVM]-x(4)-[FYH]-x(2)-E-[LIVMGA]-H-[LIVMFA](2)
[The second H is a zinc ligand]-

Note: most prokaryotic CA's as well as plant chloroplast CA's belong to another, evolutionary distinct family of proteins (see <PDOC00586

[1] Deutsch H.F. Int. J. Biochem. 19:101-113(1987).

[2] Fernley R.T. Trends Biochem. Sci. 13:356-359(1988).

[3] Tashian R.E. BioEssays 10:186-192(1989).

[4] Edwards Y. Biochem. Soc. Trans. 18:171-175(1990).

[5] Skaggs L.A., Bergenhem N.C.H., Venta P.J., Tashian R.E. Gene 126:291-292(1993).

[6] Fujiwara S., Fukuzawa H., Tachiki A., Miyachi S. Proc. Natl. Acad. Sci. U.S.A. 87:9779-9783(1990).

[7] Huang S., Xue Y., Sauer-Eriksson E., Chirica L., Lindskog S., Jonsson B.H. 2.3.CO;2-"I. Mol. Biol. 283:301-310(1998).

112. Caseins alpha/beta signature

Caseins [1] are the major protein constituent of milk. Caseins can be classified into two families; the first consists of the kappa-caseins, and the second groups the alpha-s1, alpha-s2, and beta-caseins. The alpha/beta caseins are a rapidly diverging family of proteins. However two regions are conserved: a cluster of phosphorylated serine residues and the signal sequence. The signature pattern has been developed for this family of proteins based upon the last eight residues of the signal sequence.

Consensus pattern: C-L-[LV]-A-x-A-[LVF]-A -

[1] Holt C., Sawyer L. Protein Eng. 2:251-259(1988).

113. Catalase signatures

Catalase (EC 1.11.1.6) [1,2,3] is an enzyme, present in all aerobic cells, that decomposes hydrogen peroxide to molecular oxygen and water. Its main function is to protect cells from the toxic effects of hydrogen peroxide. In eukaryotic organisms and in some prokaryotes catalase is a molecule composed of four identical subunits. Each of the subunits binds one protoheme IX group. A conserved tyrosine serves as the heme proximal side ligand. The region around this residue has been used as a first signature pattern; it also includes a conserved arginine that participates in heme-binding. A conserved histidine has been shown to be important for the catalytic mechanism of the enzyme. The region around this residue has been selected as a second signature pattern.

Consensus pattern: R-[LIVMFSTAN]-F-[GASTNP]-Y-x-D-[AST]-[QEH] [Y is the proximal heme-binding ligand]

Consensus pattern: [IF]-x-[RH]-x(4)-[EQ]-R-x(2)-H-x(2)-[GAS]-[GASTF]-[GAST] [H is an active site residue]

Note: some prokaryotic catalases belong to the peroxidase family (see <PDOC00394>).

[1] Murthy M.R.N., Reid T.J. III, Sicignano A., Tanaka N., Rossmann M.G. J. Mol. Biol. 152:465-499(1981).

[2] Melik-Adamyan W.R., Barynin V.V., Vagin A.A., Borisov V.V., Vainshtein B.K., Fita I., Murthy M.R.N., Rossmann M.G. J. Mol. Biol. 188:63-72(1986).

[3] von Ossowski I., Hausner G., Loewen P.C. J. Mol. Evol. 37:71-76(1993).

114. (chitin binding) Chitin recognition or binding domain signature

A conserved domain of 43 amino acids is found in several plant and fungal proteins that have a common binding specificity for oligosaccharides of N-acetylglucosamine [1]. This domain may be involved in the recognition or binding of chitin subunits. It has been found in the proteins listed below. - A number of non-leguminous plant lectins. The best characterized of these lectins are the three highly homologous wheat germ agglutinins (WGA-1, 2 and 3). WGA is an N-acetylglucosamine/N-acetylneuraminic acid binding lectin which structurally consists of a fourfold repetition of the 43 amino acid domain. The same type of structure is found in a barley root-specific lectin as well as a rice lectin. - Plants endochitinases (EC 3.2.1.14) from class IA (see <PDOC00620>). Endochitinases are enzymes that catalyze the hydrolysis of the beta-1,4 linkages of N-acetyl glucosamine polymers of chitin. Plant chitinases function as a defense against chitin containing fungal pathogens. Class IA

chitinases generally contain one copy of the chitin-binding domain at their N-terminal extremity. An exception is agglutinin/chitinase [2] from the stinging nettle *Urtica dioica* which contains two copies of the domain. - Hevein [5], a wound-induced protein found in the latex of rubber trees. - Win1 and win2, two wound-induced proteins from potato. -

- 5 Kluyveromyces lactis killer toxin alpha subunit [3]. The toxin encoded by the linear plasmid pGKL1 is composed of three subunits: alpha, beta, and gamma. The gamma subunit harbors toxin activity and inhibits growth of sensitive yeast strains in the G1 phase of the cell cycle; the alpha subunit, which is proteolytically processed from a larger precursor that also contains the beta subunit, is a chitinase (see <PDOC00839>). In chitinases, as well as in the

- 10 potato wound-induced proteins, the 43-residue domain directly follows the signal sequence and is therefore at the N-terminal of the mature protein; in the killer toxin alpha subunit it is located in the central section of the protein. The domain contains eight conserved cysteine residues which have all been shown, in WGA, to be involved in disulfide bonds. The topological arrangement of the four disulfide bonds is shown in the following figure: +-----

- 15 +---+ +---+ +---+ |||| xxCGxxxxxxxxCxxxxCCsxxgxCgxxxxxCxxxCxxxxC |
 *****|***** |||| +---+ +-----+ 'C': conserved cysteine involved in a
 disulfide bond. '*' : position of the pattern.

- Consensus pattern: C-x(4,5)-C-C-S-x(2)-G-x-C-G-x(4)-[FYW]-C [The five C's are involved
 20 in disulfide bonds]

[1] Wright H.T., Sandrasegaram G., Wright C.S. J. Mol. Evol. 33:283-294(1991).

[2] Lerner D.R., Raikhel N.V. J. Biol. Chem. 267:11085-11091(1992).

- [3] Butler A.R., O'Donnel R.W., Martin V.J., Gooday G.W., Stark M.J.R. Eur. J. Biochem.
 25 199:483-488(1991).

115. (Chitinase 1) Chitinases family 19 signatures

- Chitinases (EC 3.2.1.14) [1] are enzymes that catalyze the hydrolysis of the beta-1,4-N-acetyl-
 30 D-glucosamine linkages in chitin polymers. From the viewpoint of sequence similarity chitinases belong to either family 18 or 19 in the classification of glycosyl hydrolases [2,E1]. Chitinases of family 19 (also known as classes IA or I and IB or II) are enzymes from plants that function in the defense against fungal and insect pathogens by destroying their chitin-containing cell wall. Class IA/I and IB/II enzymes differ in the presence (IA/I) or absence

(IB/II) of a N-terminal chitin-binding domain (see the relevant entry <PDOC00025>). The catalytic domain of these enzymes consist of about 220 to 230 amino acid residues. Two highly conserved regions have been selected as signature patterns, the first one is located in the N-terminal section and contains one of the six cysteines which are conserved in most, if not all, of these chitinases and which is probably involved in a disulfide bond.

Consensus pattern: C-x(4,5)-F-Y-[ST]-x(3)-[FY]-[LIVMF]-x-A-x(3)-[YF]-x(2)-F- [GSA]
 Consensus pattern: [LIVM]-[GSA]-F-x-[STAG](2)-[LIVMFY]-W-[FY]-W-[LIVM]

- 10 [1] Flach J., Pilet P.-E., Jolles P. Experientia 48:701-716(1992).
- [2] Henrissat B. Biochem. J. 280:309-316(1991).

116. chloroa_b-bind

- 15 Chlorophyll A-B binding proteins. Number of members: 211

117. chromo

- The 'chromo' (CHRromatin Organization Modifier) domain [1 to 4] is a conserved region of about 60 amino acids which was originally found in Drosophila modifiers of variegation, which are proteins that modify the structure of chromatin to the condensed morphology of heterochromatin, a cytologically visible condition where gene expression is repressed. In protein Polycomb, the chromo domain has been shown to be important for chromatin targeting. Proteins that contains a chromo domain seem to fall into three classes:

- a) Proteins which have a N-terminal chromo domain followed by a region which is related to but distinct from the chromo domain and which has been termed [3] the 'chromo shadow' domain.
- 30 b) Proteins with a single chromo domain.
- c) Proteins with paired tandem chromo domains.

Currently, this domain has been found in the following proteins:

Class A.

- Drosophila heterochromatin protein Su(var)205 (HP1).
- Human heterochromatin protein HP1 alpha.
- Mammalian modifier 1 and modifier 2.
- 5 - Fission yeast swi6, a protein involved in the repression of the silent mating-type loci mat2 and mat3.

Class B.

- Drosophila protein Polycomb (Pc).
- 10 - Mammalian modifier 3, a homolog of Pc.
- Drosophila protein Su(var)3-9, a suppressor of position-effect variegation.
- Human Mi-2 autoantigen, characteristic of dermatomyositis.
- Fungal retrotransposon polyproteins: 'skippy' from *Fusarium oxysporum*, 'grasshopper' and 'MAGGY' from *Magnaporthe grisea* and CfT-1 from *Cladosporium fulvum*.
- 15 - Fission yeast hypothetical protein SpAC18G6.02c.
- *Caenorhabditis elegans* hypothetical protein C29H12.5
- *Caenorhabditis elegans* hypothetical protein ZK1236.2.
- *Caenorhabditis elegans* hypothetical protein T09A5.8.

Class C.

- Mammalian DNA-binding/helicase proteins CHD-1 to CHD-4.
- Yeast protein CHD1.
- 25 The signature pattern for this domain corresponds to its best conserved section, which is located in its central part.

-Consensus pattern: [FYL]-x-[LIVMC]-[KR]-W-x-[GDNR]-[FYWLME]-x(5,6)-[ST]-W-[ESV]-[PSTDEN]-x(2,3)-[LIVMC]

30

- [1] Paro R. Trends Genet. 6:416-421(1990).
- [2] Singh P.B., Miller J.R., Pearce J., Kothary R., Burton R.D., Paro R., James T.C., Gaunt S.J. Nucleic Acids Res. 19:789-794(1991).
- [3] Aasland R., Stewart A.F. Nucleic Acids Res. 23:3168-3173(1995).

[4] Koonin E.V., Zhou S., Lucchesi J.C. Nucleic Acids Res. 23:4229-4233(1995).

118. citrate_synt

- 5 Citrate synthase (EC 4.1.3.7) (CS) is the tricarboxylic acid cycle enzyme that catalyzes the synthesis of citrate from oxaloacetate and acetyl-CoA in an aldol condensation. CS can directly form a carbon-carbon bond in the absence of metal ion cofactors.
- 10 In prokaryotes, citrate synthase is composed of six identical subunits. In eukaryotes, there are two isozymes of citrate synthase: one is found in the mitochondrial matrix, the second is cytoplasmic. Both seem to be dimers of identical chains.
- 15 There are a number of regions of sequence similarity between prokaryotic and eukaryotic citrate synthases. One of the best conserved contains a histidine which is one of three residues shown [1] to be involved in the catalytic mechanism of the vertebrate mitochondrial enzyme. This region has been used as a signature pattern.
- 20 -Consensus pattern: G-[FYA]-[GA]-H-x-[IV]-x(1,2)-[RKT]-x(2)-D-[PS]-R [H is an active site residue]
- [1] Karpusas M., Branchaud B., Remington S.J. Biochemistry 29:2213-2219(1990).

119. clpA_B

Chaperonin clpA/B

- CAUTION! This family is a subfamily of the AAA superfamily. The threshold has been set very high to stop overlaps with the AAA superfamily. This entry will be subsumed by AAA in the future.
- 30 Number of members: 39

A number of ATP-binding proteins that are thought to protect cells from extreme stress by controlling the aggregation or denaturation of vital cellular structures have been shown [1,2] to be evolutionary related. These proteins are listed below.

5

- *Escherichia coli* clpA, which acts as the regulatory subunit of the ATP-dependent protease clp.
- *Rhodospseudomonas blastica* clpA homolog.
- *Escherichia coli* heat shock protein clpB and homologs in other bacteria.

10

- *Bacillus subtilis* protein mecB.

- Yeast heat shock protein 104 (gene HSP104), which is vital for tolerance to heat, ethanol and other stresses.

- *Neurospora* heat shock protein hsp98.

- Yeast mitochondrial heat shock protein 78 (gene HSP78) [3].

15

- CD4A and CD4b, two highly related tomato proteins that seem to be located in the chloroplast.

- *Trypanosoma brucei* protein clp.

- *Porphyra purpurea* chloroplast encoded clpC.

20

The size of these proteins range from 84 Kd (clpA) to slightly more than 100 Kd (HSP104). They all share two conserved regions of about 200 amino acids that each contains an ATP-binding site. In addition to the ATP-binding A and B motifs there are many parts in these two domains that are also conserved. Two of these regions have been selected as signature patterns. The first signature is located in the first domain, some ten residues to the C-terminal of the ATP-binding B motif. The second pattern is located in the second domain in-between the ATP-binding A and B motifs.

25

-Consensus pattern: D-[AI]-[SGA]-N-[LIVMF](2)-K-[PT]-x-L-x(2)-G

30

-Consensus pattern: R-[LIVMFY]-D-x-S-E-[LIVMFY]-x-E-[KRQ]-x-[STA]-x-[STA]-[KR]-[LIVM]-x-G-[STA]

[1] Gottesman S., Squires C., Pichersky E., Carrington M., Hobbs M., Mattick J.S., Dalrymple B., Kuramitsu H., Shiroza T., Foster T., Clark W.P., Ross B., Squires C.L., Maurizi M.R. Proc. Natl. Acad. Sci. U.S.A. 87:3513-3517(1990).

[2] Parsell D.A., Sanchez Y., Stitzel J.D., Lindquist S. Nature 353:270-273(1991).

5 [3] Leonhardt S.A., Fearon K., Danese P.N., Mason T.L. Mol. Cell. Biol. 13:6304-6313(1993).

120. cofilin_ADF

10 Cofilin/tropomyosin-type actin-binding proteins

[1]

Medline: 97290449

Structure determination of yeast cofilin.

Fedorov AA, Lappalainen P, Fedorov EV, Drubin DG, Almo SC;

15 Nat Struct Biol 1997;4:366-369.

[2]

Medline: 97290450

Crystal structure of the actin-binding protein actophorin
from Acanthamoeba.

20 Leonard SA, Gittis AG, Petrella EC, Pollard TD, Lattman EE;
Nat Struct Biol 1997;4:369-373.

[3]

Medline: 97420794

F-actin and G-actin binding are uncoupled by mutation of
25 conserved tyrosine residues in maize actin depolymerizing
factor.

Jiang CJ, Weeds AG, Khan S, Hussey PJ;

Proc Natl Acad Sci U S A 1997;94:9973-9978.

[4]

30 Medline: 97357155

Cofilin promotes rapid actin filament turnover in vivo.

Lappalainen P, Drubin DG;

Nature 1997;388:78-82.

Severs actin filaments and binds to actin monomers.

Number of members: 44

Actin-depolymerizing proteins sever actin filaments (F-actin) and/or bind to actin monomers, or G-actin, thus preventing actin-polymerization by sequestering the monomers. The following proteins are evolutionarily related and belong to a family of low molecular weight (137 to 166 residues) actin-depolymerizing proteins [1,2,3,4]:

- Cofilin from vertebrates, slime mold and yeast. Cofilin binds to F-actin and acts as a pH-dependent actin-depolymerizing protein.
- Destrin from vertebrates. Destrin binds to G-actin in a pH-independent manner and prevents polymerization.
- Caenorhabditis elegans unc-60.
- Acanthamoeba castellanii actophorin.
- Plants actin depolymerizing factor (ADF).

The most conserved region of these proteins is a twenty amino-acid segment that ends some 30 residues from their C-terminal extremity. This segment has been shown [5] to be important for actin-binding.

-Consensus pattern: P-[DE]-x-[SA]-x-[LIVMT]-[KR]-x-[KR]-M-[LIVM]-[YA]-[STA](3)-x(3)-[LIVMF]-[KR]

- [1] Hawkins M., Pope B., MacIver S.K., Weeds A.G. Biochemistry 32:9985-9993(1993).
- [2] Iida K., Moriyama K., Matsumoto S., Kawasaki H., Nishida E., Yahara I. Gene 124:115-120(1993).
- [3] Quirk S., MacIver S.K., Ampe C., Doberstein S.K., Kaiser D.A., van Damme J., Vandekerckhove J., Pollard T.D. Biochemistry 32:8525-8533(1993).
- [4] McKim K.S., Matheson C., Marra M.A., Wakarchuk M.F., Baillie D.L. Mol. Gen. Genet. 242:346-357(1994).
- [5] Moriyama K., Yonezawa N., Sakai H., Yahara I., Nishida E. J. Biol. Chem. 267:7240-7244(1992).

121. (Complex 24kd) Respiratory-chain NADH dehydrogenase 24 Kd subunit signature
Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or
NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner
mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as
5 a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this
bioenergetic enzyme complex there is one with a molecular weight of 24 Kd (in mammals),
which is a component of the iron-sulfur (IP) fragment of the enzyme. It seems to bind a 2Fe-
2S iron-sulfur cluster. The 24 Kd subunit is nuclear encoded, as a precursor form with a
transit peptide in mammals, and in *Neurospora crassa*. The 24 Kd subunit is highly similar to
10 [3,4]: - Subunit E of *Escherichia coli* NADH-ubiquinone oxidoreductase (gene *nuoE*). -
Subunit NQO2 of *Paracoccus denitrificans* NADH-ubiquinone oxidoreductase. A highly
conserved region, located in the central section of this subunit containing two conserved
cysteines that are probably involved in the binding of the 2Fe-2S center has been selected as a
signature pattern.
15 -Consensus pattern: D-x(2)-F-[ST]-x(5)-C-L-G-x-C-x(2) [GA]-P [The two C's are putative
2Fe-2S ligands]

[1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).

[2] Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).

20 [3] Fearnley I.M., Walker J.E. Biochim. Biophys. Acta 1140:105-134(1992).

[4] Weidner U., Geier S., Ptocek A., Friedrich T., Leif H., Weiss H. J. Mol. Biol. 233:109-
122(1993).

25 122. copper-bind

Copper binding proteins, plastocyanin/azurin family

Number of members: 70

Blue or 'type-1' copper proteins are small proteins which bind a single
30 copper atom and which are characterized by an intense electronic absorption
band near 600 nm [1,2]. The most well known members of this class of proteins
are the plant chloroplastic plastocyanins, which exchange electrons with
cytochrome c6, and the distantly related bacterial azurins, which exchange
electrons with cytochrome c551. This family of proteins also includes all the

proteins listed below (references are only provided for recently determined sequences).

- Amicyanin from bacteria such as *Methylobacterium extorquens* or *Thiobacillus* 5
versutus that can grow on methylamine. Amicyanin appears to be an electron
receptor for methylamine dehydrogenase.
- Auracyanins A and B from *Chloroflexus aurantiacus* [3]. These proteins can
donate electrons to cytochrome c-554.
- Blue copper protein from *Alcaligenes faecalis*.
- 10 - Cupredoxin (CPC) from cucumber peelings [4].
- Cusacyanin (basic blue protein; plantacyanin, CBP) from cucumber.
- Halocyanin from *Natrobacterium pharaonis* [5], a membrane associated copper-
binding protein.
- Pseudoazurin from *Pseudomonas*.
- 15 - Rusticyanin from *Thiobacillus ferrooxidans*. Rusticyanin is an electron
carrier from cytochrome c-552 to the a-type oxidase [6].
- Stellacyanin from the Japanese lacquer tree.
- Umecyanin from horseradish roots.
- 20 - Allergen Ra3 from ragweed. This pollen protein is evolutionary related to
the above proteins, but seems to have lost the ability to bind copper.

Although there is an appreciable amount of divergence in the sequence of all
these proteins, the copper ligand sites are conserved and a pattern which includes two 25
of the ligands (a cysteine and a histidine) has been developed.

-Consensus pattern: [GA]-x(0,2)-[YSA]-x(0,1)-[VFY]-x-C-x(1,2)-[PG]-x(0,1)-H-x(2,4)-
[MQ] [C and H are copper ligands]

- 30 [1] Garret T.P.J., Clingeffer D.J., Guss J.M., Rogers S.J., Freeman H.C. J. Biol. Chem.
259:2822-2825(1984).
- [2] Ryden L.G., Hunt L.T. J. Mol. Evol. 36:41-66(1993).
- [3] McManus J.D., Brune D.C., Han J., Sanders-Loehr J., Meyer T.E., Cusanovich M.A.,
Tollin G., Blankenship R.E. J. Biol. Chem. 267:6531-6540(1992).

[4] Mann K., Schaefer W., Thoenes U., Messerschmidt A., Mehrabian Z., Nalbandyan R. FEBS Lett. 314:220-223(1992).

[5] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

5 [6] Yano T., Fukumori Y., Yamanaka T. FEBS Lett. 288:159-162(1991).

123. Chaperonins cpn10 signature

Chaperonins [1,2] are proteins involved in the folding of proteins or the assembly of
 10 oligomeric protein complexes. They seem to assist other polypeptides in maintaining or
 assuming conformations which permit their correct assembly into oligomeric structures. They
 are found in abundance in prokaryotes, chloroplasts and mitochondria. Chaperonins form
 oligomeric complexes and are composed of two different types of subunits: a 60 Kd protein,
 known as cpn60 (groEL in bacteria) and a 10 Kd protein, known as cpn10 (groES in
 15 bacteria).The cpn10 protein binds to cpn60 in the presence of MgATP and suppresses the
 ATPase activity of the latter. Cpn10 is a protein of about 100 amino acid residues whose
 sequence is well conserved in bacteria, vertebrate mitochondria and plants chloroplast [3,4].
 Cpn10 assembles as an heptamer that forms a dome[5]. As a signature pattern for cpn10 , a
 region located in the N-terminal section of the protein was selected.

20 Consensus pattern: [LIVMFY]-x-P-[ILT]-x-[DEN]-[KR]-[LIVMFA](3)-[KREQ]-x(8,9)-
 [SG]-x-[LIVMFY](3)-

Note: this pattern is found twice in the plant chloroplast protein which consist of the tandem
 repeat of a cpn10 domain

25 [1] Ellis R.J., van der Vies S.M. Annu. Rev. Biochem. 60:321-347(1991).

[2] Zeilstra-Ryalls J., Fayet O., Georgopoulos C. Annu. Rev. Microbiol. 45:301-325(1991).

[3] Hartman D.J., Hoogenraad N.J., Condon R., Hoj P.B. Proc. Natl. Acad. Sci. U.S.A. 89:3394-3398(1992).

30 [4] Bertsch U., Soll J., Seetharam R., Viitanen P.V. Proc. Natl. Acad. Sci. U.S.A. 89:8696-8700(1992).

[5] Hunt J.F., Weaver A.J., Landry S.J., Gierasch L., Deisenhofer J. Nature 379:37-45(1996).

124. Chaperonins cpn60 signature (cpn60_TCP1)

Chaperonins [1,2] are proteins involved in the folding of proteins or the assembly of oligomeric protein complexes. Their role seems to be to assist other polypeptides to maintain or assume conformations which permit their correct assembly into oligomeric structures.

- 5 They are found in abundance in prokaryotes, chloroplasts and mitochondria. Chaperonins form oligomeric complexes and are composed of two different types of subunits: a 60 Kd protein, known as cpn60 (groEL in bacteria) and a 10 Kd protein, known as cpn10 (groES in bacteria). The cpn60 protein shows weak ATPase activity and is a highly conserved protein of about 550 to 580 amino acid residues which has been described by different names in
- 10 different species: - *Escherichia coli* groEL protein, which is essential for the growth of the bacteria and the assembly of several bacteriophages. - Cyanobacterial groEL analogues. - *Mycobacterium tuberculosis* and *leprae* 65 Kd antigen, *Coxiella burnetti* heat shock protein B (gene htpB), *Rickettsia tsutsugamushi* major antigen 58, and *Chlamydia* 57 Kd hypersensitivity antigen (gene hypB). - Chloroplast RuBisCO subunit binding-protein alpha and beta chains, which bind ribulose biphosphate carboxylase small and large subunits and
- 15 are implicated in the assembly of the enzyme oligomer. - Mammalian mitochondrial matrix protein P1 (mitonin or P60). - Yeast HSP60 protein, a mitochondrial assembly factor. As a signature pattern for these proteins, a rather well-conserved region of twelve residues, located in the last third of the cpn60 sequence was chosen.

20 Consensus pattern: A-[AS]-x-[DEQ]-E-x(4)-G-G-[GA]-

[1] Ellis R.J., van der Vies S.M. Annu. Rev. Biochem. 60:321-347(1991).

[2] Zeilstra-Ryalls J., Fayet O., Georgopoulos C. Annu. Rev. Microbiol. 45:301-325(1991).

25 Chaperonins TCP-1 signatures (cpn60_TCP1)

The TCP-1 protein [1,2] (Tailless Complex Polypeptide 1) was first identified in mice where it is especially abundant in testis but present in all cell types. It has since been found and characterized in many other mammalian species, in *Drosophila* and in yeast. TCP-1 is a

30 highly conserved protein of about 60 Kd (556 to 560 residues) which participates in a hetero-oligomeric 900 Kd double-torus shaped particle [3] with 6 to 8 other different subunits. These subunits, the chaperonin containing TCP-1 (CCT) subunit beta, gamma, delta, epsilon, zeta and eta are evolutionary related to TCP-1 itself [4,5]. The CCT is known to act as a molecular chaperone for tubulin, actin and probably some other proteins. The CCT subunits are highly

related to archeobacterial counterparts: - TF55 and TF56 [6], a molecular chaperone from *Sulfolobus shibatae*. TF55 has ATPase activity, is known to bind unfolded polypeptides and forms a oligomeric complex of two stacked nine-membered rings. - Thermosome [7], from *Thermoplasma acidophilum*. The thermosome is composed of two subunits (alpha and beta) and also seems to be a chaperone with ATPase activity. It forms an oligomeric complex of eight-membered rings. The TCP-1 family of proteins are weakly, but significantly [8], related to the cpn60/groEL chaperonin family (see <PDOC00268>). As signature patterns of this family of chaperonins, three conserved regions located in the N-terminal domain were chosen.

Consensus pattern: [RKEL]-[ST]-x-[LMFY]-G-P-x-[GSA]-x-x-K-[LIVMF](2)-

Consensus pattern: [LIVM]-[TS]-[NK]-D-[GA]-[AVNHK]-[TAV]-[LIVM](2)-x(2)-
[LIVM]-x-[LIVM]-x-[SNH]-[POH]-

Consensus pattern: Q-[DEK]-x-x-[LIVMGTA]-[GA]-D-G-T-

[1] Ellis J. Nature 358:191-192(1992).

[2] Nelson R.J., Craig E.A. Curr. Biol. 2:487-489(1992).

[3] Lewis V.A., Hynes G.M., Zheng D., Saibil H., Willison K.R. Nature 358:249-252(1992).

[4] Kubota H., Hynes G., Carne A., Ashworth A., Willison K.R. Curr. Biol. 4:89-99(1994)

[5] Kim S., Willison K.R., Horwich A.L. Trends Biochem. Sci. 20:543-548(1994).

[6] Trent J.D., Nimmesgern E., Wall J.S., Hartl F.U., Horwich A.L. Nature 354:490-493(1991).

[7] Waldmann T., Lupas A., Kellermann J., Peters J., Baumeister W. Biol. Chem. Hoppe-Seyler 376:119-126(1995).

[8] Hemmingsen S.M. Nature 357:650-650(1992).

125. cyclin (Cyclins)

The cyclins include an internal duplication, which is related to that found in TFIIIB and the RB protein.

[1]

Medline: 94203808

Evidence for a protein domain superfamily shared by the cyclins, TFIIIB and RB/p107.

Gibson TJ, Thompson JD, Blocker A, Kouzarides T;
Nucleic Acids Res 1994;22:946-952.

[2]

Medline: 96164440

5 The crystal structure of cyclin A

Brown NR, Noble MEM, Endicott JA, Garman EF, Wakatsuki S,
Mitchell E, Rasmussen B, Hunt T, Johnson LN;
Structure. 1995;3:1235-1247.

Complex of cyclin and cyclin dependant kinase.

10 [3]

Medline: 96313126

Structural basis of cyclin-dependant kinase activation by
phosphorylation.

Russo AA, Jeffrey PD, Pavletich NP;

15 Nat Struct Biol. 1996;3:696-700.

Cyclins regulate cyclin dependant kinases (CDKs).

The most divergent prosite members have been included. Swiss:P22674
the Uracil-DNA glycosylase 2 is the highest noise and may be related
but has not been included.

20 Number of members: 189

Cyclins [1,2,3] are eukaryotic proteins which play an active role in
controlling nuclear cell division cycles. Cyclins, together with the p34
(cdc2) or cdk2 kinases, form the Maturation Promoting Factor (MPF). There are

25 two main groups of cyclins:

- G2/M cyclins, essential for the control of the cell cycle at the G2/M
(mitosis) transition. G2/M cyclins accumulate steadily during G2 and are
abruptly destroyed as cells exit from mitosis (at the end of the M-phase).

30 - G1/S cyclins, essential for the control of the cell cycle at the G1/S
(start) transition.

In most species, there are multiple forms of G1 and G2 cyclins. For example,
in vertebrates, there are two G2 cyclins, A and B, and at least three G1

cyclins, C, D, and E.

A cyclin homolog has also been found in herpesvirus saimiri [4].

- 5 The best conserved region is in the central part of the cyclins' sequences, known as the 'cyclin-box'. From this, a 32 residue pattern has been derived.

-Consensus pattern: R-x(2)-[LIVMSA]-x(2)-[FYWS]-[LIVM]-x(8)-[LIVMFC]-x(4)-
[LIVMFYA]-x(2)-[STAGC]-[LIVMFYQ]-x-[LIVMFYC]-[LIVMFY]-D-[RKH]-
10 [LIVMFYW]

[1] Nurse P. Nature 344:503-508(1990).

[2] Norbury C., Nurse P. Curr. Biol. 1:23-24(1991).

[3] Lew D.J., Reed S.I. Trends Cell Biol. 2:77-81(1992).

- 15 [4] Nicholas J., Cameron K.R., Honess R.W. Nature 355:362-365(1992).

126. Cystatin domain

- 20 This is a very diverse family. Attempts to define separate subfamilies have failed. Typically, either the N-terminal or C-terminal end is very divergent. But splitting into two domains would make very short families. Cathelicidins are related to this family but have not been included. Number of members: 147

- 25 Inhibitors of cysteine proteases [1,2,3], which are found in the tissues and body fluids of animals, in the larva of the worm *Onchocerca volvulus* [4], as well as in plants, can be grouped into three distinct but related families:

- Type 1 cystatins (or stefins), molecules of about 100 amino acid residues with neither disulfide bonds nor carbohydrate groups.
- Type 2 cystatins, molecules of about 115 amino acid residues which contain one or two disulfide loops near their C-terminus.
- 30 - Kininogens, which are multifunctional plasma glycoproteins.

They are the precursor of the active peptide bradykinin and play a role in blood coagulation by helping to position optimally prekallikrein and factor XI next to factor XII. They are also inhibitors of cysteine proteases. Structurally, kininogens are made of three contiguous type-2 cystatin domains, followed by an additional domain (of variable length)

which contains the sequence of bradykinin. The first of the three cystatin domains seems to have lost its inhibitory activity.

In all these inhibitors, there is a conserved region of five residues which has been proposed to be important for the binding to the cysteine proteases. The consensus pattern starts one residue before this conserved region.

-Consensus pattern: [GSTEQKRV]-Q-[LIVT]-[VAF]-[SAGQ]-G-x-[LIVMNK]-x(2)-[LIVMFY]-x-[LIVMFYA]-[DENQKRHSIV]

[1] Barrett A.J. Trends Biochem. Sci. 12:193-196(1987).

[2] Rawlings N.D., Barrett A.J. J. Mol. Evol. 30:60-71(1990).

[3] Turk V., Bode W. FEBS Lett. 285:213-219(1991).

[4] Lustigman S., Brotman B., Huima T., Prince A.M. Mol. Biochem. Parasitol. 45:65-76(1991).

127. cytochrome_c (Cytochrome c)

The Pfam entry does not include all prosite members.

The cytochrome 556 and cytochrome c' families are not included.

Number of members: 259

In proteins belonging to cytochrome c family [1], the heme group is covalently attached by thioether bonds to two conserved cysteine residues. The consensus sequence for this site is Cys-X-X-Cys-His and the histidine residue is one of the two axial ligands of the heme iron. This arrangement is shared by all proteins known to belong to cytochrome c family, which presently includes cytochromes c, c', c1 to c6, c550 to c556, cc3/Hmc, cytochrome f and reaction center cytochrome c.

-Consensus pattern: C-{CPWHF}-{CPWR}-C-H-{CFYW}

[1] Mathews F.S. Prog. Biophys. Mol. Biol. 45:1-56(1985).

128. (DAGKa) Diacylglycerol kinase accessory domain (presumed)

Diacylglycerol (DAG) is a second messenger that acts as a protein kinase C activator. This domain is assumed to be an accessory domain: its function is unknown.

[1] Sakane F, Yamada K, Kanoh H, Yokoyama C, Tanabe T, Nature 1990;344:345-348. [2] Sakane F, Imai S, Kai M, Wada I, Kanoh H, J Biol Chem 1996;271:8394-8401. [3] Schaap D, de Widt J, van der Wal J, Vandekerckhove J, van, Damme J, Gussow D, Ploegh HL, van Blitterswijk WJ, van der, Bend RL, FEBS Lett 1990;275:151-158. [4] Kanoh H, Yamada K, Sakane F, Trends Biochem Sci 1990;15:47-50.

129. (DAGKc) Diacylglycerol kinase catalytic domain (presumed)

Diacylglycerol (DAG) is a second messenger that acts as a protein kinase C activator. The catalytic domain is assumed from the finding of bacterial homologues.

[1] Sakane F, Yamada K, Kanoh H, Yokoyama C, Tanabe T, Nature 1990;344:345-348. [2] Sakane F, Imai S, Kai M, Wada I, Kanoh H, J Biol Chem 1996;271:8394-8401. [3] Schaap D, de Widt J, van der Wal J, Vandekerckhove J, van, Damme J, Gussow D, Ploegh HL, van Blitterswijk WJ, van der, Bend RL, FEBS Lett 1990;275:151-158. [4] Kanoh H, Yamada K, Sakane F, Trends Biochem Sci 1990;15:47-50.

130. D-amino acid oxidases signature(DAO)

D-amino acid oxidase (EC 1.4.3.3) (DAMOX or DAO) is an FAD flavoenzyme that catalyzes the oxidation of neutral and basic D-amino acids into their corresponding keto acids. DAOs have been characterized and sequenced in fungi and vertebrates where they are known to be located in the peroxisomes. D-aspartate oxidase (EC 1.4.3.1) (DASOX) [1] is an enzyme, structurally related to DAO, which catalyzes the same reaction but is active only toward dicarboxylic D-amino acids. In DAO, a conserved histidine has been shown [2] to be important for the enzyme's catalytic activity. The conserved region around this residue has been developed as a signature pattern for these enzymes.

Consensus pattern: [LIVM](2)-H-[NHA]-Y-G-x-[GSA](2)-x-G-x(5)-G-x-A [H is a probable active site residue]o-

- [1] Negri A., Ceciliani F., Tedeschi G., Simonic T., Ronchi S. J. Biol. Chem. 267:11865-11871(1992).
- [2] Miyano M., Fukui K., Watanabe F., Takahashi S., Tada M., Kanashiro M., Miyake Y. J. Biochem. 109:171-177(1991).

5

131. DEAD and DEAH box families ATP-dependent helicases signatures

A number of eukaryotic and prokaryotic proteins have been characterized [1,2,3] on the basis of their structural similarity. They all seem to be involved in ATP-dependent, nucleic-acid unwinding. Proteins currently known to belong to this family are: - Initiation factor eIF-4A. Found in eukaryotes, this protein is a subunit of a high molecular weight complex involved in 5'cap recognition and the binding of mRNA to ribosomes. It is an ATP-dependent RNA-helicase. - PRP5 and PRP28. These yeast proteins are involved in various ATP-requiring steps of the pre-mRNA splicing process. - P110, a mouse protein expressed specifically during spermatogenesis. - An3, a *Xenopus* putative RNA helicase, closely related to P110. - SPP81/DED1 and DBP1, two yeast proteins probably involved in pre-mRNA splicing and related to P110. - *Caenorhabditis elegans* helicase glh-1. - MSS116, a yeast protein required for mitochondrial splicing. - SPB4, a yeast protein involved in the maturation of 25S ribosomal RNA. - p68, a human nuclear antigen. p68 has ATPase and DNA-helicase activities in vitro. It is involved in cell growth and division. - Rm62 (p62), a *Drosophila* putative RNA helicase related to p68. - DBP2, a yeast protein related to p68. - DHH1, a yeast protein. - DRS1, a yeast protein involved in ribosome assembly. - MAK5, a yeast protein involved in maintenance of dsRNA killer plasmid. - ROK1, a yeast protein. - ste13, a fission yeast protein. - Vasa, a *Drosophila* protein important for oocyte formation and specification of embryonic posterior structures. - Me31B, a *Drosophila* maternally expressed protein of unknown function. - dbpA, an *Escherichia coli* putative RNA helicase. - deaD, an *Escherichia coli* putative RNA helicase which can suppress a mutation in the rpsB gene for ribosomal protein S2. - rhlB, an *Escherichia coli* putative RNA helicase. - rhlE, an *Escherichia coli* putative RNA helicase. - smnB, an *Escherichia coli* protein that shows RNA-dependent ATPase activity. It probably interacts with 23S ribosomal RNA. - *Caenorhabditis elegans* hypothetical proteins T26G10.1, ZK512.2 and ZK686.2. - Yeast hypothetical protein YHR065c. - Yeast hypothetical protein YHR169w. - Fission yeast hypothetical protein SpAC31A2.07c. - *Bacillus subtilis* hypothetical protein yxiN. All these proteins share a number of conserved sequence motifs. Some of them are specific to this family while others

30

are shared by other ATP-binding proteins or by proteins belonging to the helicases 'superfamily' [4,E1]. One of these motifs, called the 'D-E-A-D-box', represents a special version of the B motif of ATP-binding proteins. Some other proteins belong to a subfamily which have His instead of the second Asp and are thus said to be 'D-E-A-H-box' proteins

5 [3,5,6,E1]. Proteins currently known to belong to this subfamily are: - PRP2, PRP16, PRP22 and PRP43. These yeast proteins are all involved in various ATP-requiring steps of the pre-mRNA splicing process. - Fission yeast prh1, which may be involved in pre-mRNA splicing. - Male-less (mle), a Drosophila protein required in males, for dosage compensation of X chromosome linked genes. - RAD3 from yeast. RAD3 is a DNA helicase involved in excision

10 repair of DNA damaged by UV light, bulky adducts or cross-linking agents. Fission yeast rad15 (rhp3) and mammalian DNA excision repair protein XPD (ERCC-2) are the homologs of RAD3. - Yeast CHL1 (or CTF1), which is important for chromosome transmission and normal cell cycle progression in G(2)/M. - Yeast TPS1. - Yeast hypothetical protein YKL078w. - Caenorhabditis elegans hypothetical proteins C06E1.10 and K03H1.2. -

15 Poxviruses' early transcription factor 70 Kd subunit which acts with RNA polymerase to initiate transcription from early gene promoters. - I8, a putative vaccinia virus helicase. - hrpA, an Escherichia coli putative RNA helicase. Signature patterns for both subfamilies were developed.

20 Consensus pattern: [LIVMF](2)-D-E-A-D-[RKEN]-x-[LIVMFYGSTN]

Consensus pattern: [GSAH]-x-[LIVMF](3)-D-E-[ALIV]-H-[NECR]

Note: proteins belonging to this family also contain a copy of the ATP/GTP- binding motif 'A' (P-loop) (see the relevant entry <PDOC00017

25 [1] Schmid S.R., Linder P. Mol. Microbiol. 6:283-292(1992).

[2] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).

[3] Wassarman D.A., Steitz J.A. Nature 349:463-464(1991).

[4] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).

30 [5] Harosh I., Deschavanne P. Nucleic Acids Res. 19:6331-6331(1991).

[6] Koonin E.V., Senkevich T.G. J. Gen. Virol. 73:989-993(1992).

3,4-Dihydroxy-2-butanone 4-phosphate is biosynthesized from ribulose 5-phosphate and serves as the biosynthetic precursor for the xylene ring of riboflavin. Sometimes found as a bifunctional enzyme with GTP_cyclohydro2.

Richter G, Krieger C, Volk R, Kis K, Ritz H, Gotze E, Bacher A, Methods Enzymol
5 1997;280:374-382.

133. (DHDPS) Dihydrodipicolinate synthetase signatures

Dihydrodipicolinate synthetase (EC 4.2.1.52) (DHDPS) [1] catalyzes, in higher plants
10 chloroplast and in many bacteria (gene dapA), the first reaction specific to the biosynthesis of lysine and of diaminopimelate. DHDPS is responsible for the condensation of aspartate semialdehyde and pyruvate by aping-pong mechanism in which pyruvate first binds to the enzyme by forming a Schiff-base with a lysine residue. Three other proteins are structurally related to DHDPS and probably also act via a similar catalytic mechanism: - Escherichia coli
15 N-acetylneuraminase lyase (EC 4.1.3.3) (gene nanA), which catalyzes the condensation of N-acetyl-D-mannosamine and pyruvate to form N-acetylneuraminate. - Rhizobium meliloti protein mosA [3], which is involved in the biosynthesis of the rhizopine 3-o-methyl-scylo-inosamine. - Escherichia coli hypothetical protein yjhH. Two signature patterns for these
20 enzymes were developed. The first one is centered on highly conserved region in the N-terminal part of these proteins. The second signature contains a lysine residue which has been shown, in Escherichia coli dapA [2], to be the one that forms a Schiff-base with the substrate.

Consensus pattern: [GSA]-[LIVM]-[LIVMFY]-x(2)-G-[ST]-[TG]-G-E-[GASNF]-x(6)- [EQ]

25 Consensus pattern: Y-[DNS]-[LIVMFA]-P-x(2)-[ST]-x(3)-[LIVMG]-x(13,14)-[LIVM]- x-[SGA]-[LIVMF]-K-[DEQAF]-[STAC] [K is involved in Schiff-base formation]-

[1] Kaneko T., Hashimoto T., Kumpaisal R., Yamada Y. J. Biol. Chem. 265:17451-17455(1990).

30 [2] Laber B., Gomis-Rueth F.-X., Romao M.J., Huber R. Biochem. J. 288:691-695(1992).

[3] Murphy P.J., Trenz S.P., Grzemeski W., de Bruijn F.J., Schell J. J. Bacteriol. 175:5193-5204 (1993).

134. (DHodehase) Dihydroorotate dehydrogenase signatures

Dihydroorotate dehydrogenase (EC 1.3.3.1) (DHodehase) catalyzes the fourth step in the de novo biosynthesis of pyrimidine, the conversion of dihydroorotate into orotate. DHodehase is a ubiquitous FAD flavoprotein. In bacteria (gene *pyrD*), DHodehase is located on the inner side of the cytosolic membrane. In some yeasts, such as in *Saccharomyces cerevisiae* (gene *URA1*), it is a cytosolic protein while in other eukaryotes it is found in the mitochondria [1]. The sequence of DHodehase is rather well conserved and two signature patterns were developed specific to this enzyme. The first corresponds to a region in the N-terminal section of the enzyme while the second is located in the C-terminal section and seems to be part of the FAD-binding domain.

Consensus pattern[GS]-x(4)-[GK]-[GSTA]-[LIVFSTA]-[GT]-x(3)-[NQR]-x-G-[NHY]-x(2)-P-[RT]

Consensus pattern[LIVM](2)-[GSA]-x-G-G-[IV]-x-[STGDN]-x(3)-[ACV]-x(6)-G-A

[1] Nagy M., Lacroute F., Thomas D. Proc. Natl. Acad. Sci. U.S.A. 89:8966-8970(1992).

135. (DMRL_synthase) 6,7-dimethyl-8-ribityllumazine synthase

136. (DNA_methylase) C-5 cytosine-specific DNA methylases signatures

C-5 cytosine-specific DNA methylases (EC 2.1.1.73) (C5 Mtase) are enzymes that specifically methylate the C-5 carbon of cytosines in DNA [1,2,3]. Such enzymes are found in the proteins described below. - As a component of type II restriction-modification systems in prokaryotes and some bacteriophages. Such enzymes recognize a specific DNA sequence where they methylate a cytosine. In doing so, they protect DNA from cleavage by type II restriction enzymes that recognize the same sequence. The sequences of a large number of type II C-5 Mtases are known. - In vertebrates, there are a number of C-5 Mtases that methylate CpG dinucleotides. The sequence of the mammalian enzyme is known. C-5 Mtases share a number of short conserved regions. Two of them were selected. The first is centered around a conserved Pro-Cys dipeptide in which the cysteine has been shown [4] to be involved in the catalytic mechanism; it appears to form a covalent intermediate with the C6

position of cytosine. The second region is located at the C-terminal extremity in type-II enzymes

Consensus pattern: [DENKS]-x-[FLIV]-x(2)-[GSTC]-x-P-C-x(2)-[FYWLLIM]-S [C is the active site residue]-

Consensus pattern: [RKQGTf]-x(2)-G-N-[STAG]-[LIVMF]-x(3)-[LIVMT]-x(3)-[LIVM]-x(3)-[LIVM]-

[1] Posfai J., Bhagwat A.S., Roberts R.J. Gene 74:261-263(1988).

[2] Kumar S., Cheng X., Klimasauskas S., Mi S., Posfai J., Roberts R.J., Wilson G.G. Nucleic Acids Res. 22:1-10(1994).

[3] Lauster R., Trautner T.A., Noyer-Weidner M. J. Mol. Biol. 206:305-312(1989).

[4] Chen L., McMillan A.M., Chang W., Ezak-Nipkay K., Lane W.S., Verdine G.L. Biochemistry 30:11018-11025(1991).

137. (DNAphotolyase) DNA photolyases class 2 signatures

Deoxyribodipyrimidine photolyase (EC 4.1.99.3) (DNA photolyase) [1,2] is a DNArepair enzyme. It binds to UV-damaged DNA containing pyrimidine dimers and, upon absorbing a near-UV photon (300 to 500 nm), breaks the cyclobutane ring joining the two pyrimidines of the dimer. DNA photolyase is an enzyme that requires two chromophore-cofactors for its activity: a reduced FADH2 and either 5,10-methenyltetrahydrofolate (5,10-MTFH) or an oxidized 8-hydroxy-5-deazaflavin (8-HDF) derivative (F420). The folate or deazaflavin chromophore appears to function as an antenna, while the FADH2 chromophore is thought to be responsible for electron transfer. On the basis of sequence similarities[3] DNA photolyases can be grouped into two classes. The second class contains enzymes from *Myxococcus xanthus*, methanogenic archaeobacteria, insects, fish and marsupial mammals. It is not yet known what second cofactor is bound to class 2 enzymes. There are a number of conserved sequence regions in all known class 2 DNAphotolyases, especially in the C-terminal part. Two of these regions were selected as signature patterns.

Consensus pattern: F-x-E-E-x-[LIVM](2)-R-R-E-L-x(2)-N-F-

Consensus pattern: G-x-H-D-x(2)-W-x-E-R-x-[LIVM]-F-G-K-[LIVM]-R-[FY]-M-N-

[1] Sancar G.B., Sancar A. Trends Biochem. Sci. 12:259-261(1987).

[2] Jorns M.S. Biofactors 2:207-211(1990).

[3] Yasui A., Eker A.P.M., Yasuhira S., Yajima H., Kobayashi T., Takao M., Oikawa A.
EMBO J. 13:6143-6151(1994).

5 (DNPhotolyase2) DNA photolyases class 1 signatures

Deoxyribodipyrimidine photolyase (EC 4.1.99.3) (DNA photolyase) [1,2] is a DNA repair enzyme. It binds to UV-damaged DNA containing pyrimidine dimers and, upon absorbing a near-UV photon (300 to 500 nm), breaks the cyclobutane ring joining the two pyrimidines of the dimer. DNA photolyase is an enzyme that requires two chromophore-cofactors for its activity: a reduced FADH2 and either 5,10-methenyltetrahydrofolate (5,10-MTHF) or an oxidized 8-hydroxy-5-deazaflavin (8-HDF) derivative (F420). The folate or deazaflavin chromophore appears to function as an antenna, while the FADH2 chromophore is thought to be responsible for electron transfer. On the basis of sequence similarities[3] DNA photolyases can be grouped into two classes. The first class contains enzymes from Gram-negative and Gram-positive bacteria, the halophilic archaeobacteria Halobacterium halobium, fungi and plants. Class 1 enzymes bind either 5,10-MTHF (E.coli, fungi, etc.) or 8-HDF (S.griseus, H.halobium). This family also includes Arabidopsis cryptochromes 1 (CRY1) and 2 (CRY2), which are blue light photoreceptors that mediate blue light-induced gene expression. There are a number of conserved sequence regions in all known class 1 DNA photolyases, especially in the C-terminal part. Two of these regions were selected as signature patterns

Consensus pattern: T-G-x-P-[LIVM](2)-D-A-x-M-[RA]-x-[LIVM]-

Consensus pattern: [DN]-R-x-R-[LIVM](2)-x-[STA](2)-F-[LIVMFA]-x-K-x-L-x(2,3)-W-[KRO]-

[1] Sancar G.B., Sancar A. Trends Biochem. Sci. 12:259-261(1987).

[2] Jorns M.S. Biofactors 2:207-211(1990).

[3] Yasui A., Eker A.P.M., Yasuhira S., Yajima H., Kobayashi T., Takao M., Oikawa A.
EMBO J. 13:6143-6151(1994).

[4] Lin C., Ahmad M., Cashmore A.R. Plant J. 10:893-902(1996).

DNA polymerase family A signature

Replicative DNA polymerases (EC 2.7.7.7) are the key enzymes catalyzing the accurate replication of DNA. They require either a small RNA molecule or a protein as a primer for the de novo synthesis of a DNA chain. On the basis of sequence similarities a number of

- 5 DNA polymerases have been grouped together [1,2,3] under the designation of DNA polymerase family A. The polymerases that belong to this family are listed below.

- Escherichia coli and various other bacterial polymerase I (gene polA).
- Thermus aquaticus Taq polymerase.
- 10 - Bacteriophage sp01 polymerase.
- Bacteriophage sp02 polymerase.
- Bacteriophage T5 polymerase.
- Bacteriophage T7 polymerase.
- Mycobacteriophage L5 polymerase.
- 15 - Yeast mitochondrial polymerase gamma (gene MIP1).

Five regions of similarity are found in all the above polymerases. One of these conserved regions, known as 'motif B' [1], is located in a domain which, in Escherichia coli polA, has been shown to bind deoxynucleotide triphosphate substrates; it contains a conserved tyrosine
20 which has been shown, by photo- affinity labelling, to be in the active site; a conserved lysine, also part of this motif, can be chemically labelled, using pyridoxal phosphate. This conserved region was used as a signature for this family of DNA polymerases.

Consensus pattern R-x(2)-[GSAV]-K-x(3)-[LIVMFY]-[AGQ]-x(2)-Y-x(2)-[GS]-x(3)-

- 25 [LIVMA] Sequences known to belong to this class detected by the pattern ALL.

[1] Delarue M., Poch O., Todro N., Moras D., Argos P. Protein Eng. 3:461-467(1990).

[2] Ito J., Braithwaite D.K. Nucleic Acids Res. 19:4045-4057(1991).

[3] Braithwaite D.K., Ito J. Nucleic Acids Res. 21:787-802(1993).

30 139. DNA_pol_viral_C

DNA polymerase (viral) C-terminal domain

Number of members: 128

140. (DNA_topoisomII)

DNA topoisomerase II signature

- 5 DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type II topoisomerases are ATP-dependent and act by passing a DNA segment through a transient double-strand break. Topoisomerase II is found in phages, archaebacteria, prokaryotes, eukaryotes, and in African Swine Fever virus (ASF). In bacteriophage T4 topoisomerase II consists of three subunits
- 10 (the product of genes 39, 52 and 60). In prokaryotes and in archaebacteria the enzyme, known as DNA gyrase, consists of two subunits (genes *gyrA* and *gyrB* [E2]). In some bacteria, a second type II topoisomerase has been identified; it is known as topoisomerase IV and is required for chromosome segregation, it also consists of two subunits (genes *parC* and *parE*). In eukaryotes, type II topoisomerase is a homodimer.

15 There are many regions of sequence homology between the different subtypes of topoisomerase II. The relation between the different subunits is shown in the following representation:

20 <-----About-1400-residues----->

[-----Protein 39-*-----][----Protein 52----] Phage T4

[-----gyrB-----*-----][-----gyrA-----] Prokaryote II

Archaebacteria

25 [-----parE-----*-----][-----parD-----] Prokaryote IV

[-----*-----] Eukaryote and

ASF

*: Position of the pattern.

- 30 As a signature pattern for this family of proteins, a region that contains a highly conserved pentapeptide was selected. The pattern is located in *gyrB*, in *parE*, and in protein 39 of phage T4 topoisomerase.

Consensus pattern[LIVMA]-x-E-G-[DN]-S-A-x-[STAG] Sequences known to belong to this class detected by the pattern ALL.

[1] Sternglanz R. Curr. Opin. Cell Biol. 1:533-535(1990).

5 [2] Bjornsti M.-A. Curr. Opin. Struct. Biol. 1:99-103(1991).

[3] Sharma A., Mondragon A. Curr. Opin. Struct. Biol. 5:39-47(1995).

[4] Roca J. Trends Biochem. Sci. 20:156-160(1995).

10 141. (DSPc) Tyrosine specific protein phosphatases signature and profiles

Tyrosine specific protein phosphatases (EC 3.1.3.48) (PTPase) [1 to 5] are enzymes that catalyze the removal of a phosphate group attached to a tyrosine residue. These enzymes are very important in the control of cell growth, proliferation, differentiation and transformation. Multiple forms of PTPase have been characterized and can be classified into two categories:

- 15 soluble PTPases and transmembrane receptor proteins that contain PTPase domain(s). The currently known PTPases are listed below: Soluble PTPases. - PTPN1 (PTP-1B). - PTPN2 (T-cell PTPase; TC-PTP). - PTPN3 (H1) and PTPN4 (MEG), enzymes that contain an N-terminal band 4.1- like domain (see <PDOC00566>) and could act at junctions between the membrane and cytoskeleton. - PTPN5 (STEP). - PTPN6 (PTP-1C; HCP; SHP) and PTPN11
- 20 (PTP-2C; SH-PTP3; Syp), enzymes which contain two copies of the SH2 domain at its N-terminal extremity. The Drosophila protein corkscrew (gene csw) also belongs to this subgroup. - PTPN7 (LC-PTP; Hematopoietic protein-tyrosine phosphatase; HePTP). - PTPN8 (70Z-PEP). - PTPN9 (MEG2). - PTPN12 (PTP-G1; PTP-P19). - Yeast PTP1. - Yeast PTP2 which may be involved in the ubiquitin-mediated protein degradation pathway. -
- 25 Fission yeast pyp1 and pyp2 which play a role in inhibiting the onset of mitosis. - Fission yeast pyp3 which contributes to the dephosphorylation of cdc2. - Yeast CDC14 which may be involved in chromosome segregation. - Yersinia virulence plasmid PTPases (gene yopH). - Autographa californica nuclear polyhedrosis virus 19 Kd PTPase. Dual specificity PTPases. - DUSP1 (PTPN10; MAP kinase phosphatase-1; MKP-1); which dephosphorylates MAP
- 30 kinase on both Thr-183 and Tyr-185. - DUSP2 (PAC-1), a nuclear enzyme that dephosphorylates MAP kinases ERK1 and ERK2 on both Thr and Tyr residues. - DUSP3 (VHR). - DUSP4 (HVH2). - DUSP5 (HVH3). - DUSP6 (Pyst1; MKP-3). - DUSP7 (Pyst2; MKP-X). - Yeast MSG5, a PTPase that dephosphorylates MAP kinase FUS3. - Yeast YVH1. - Vaccinia virus H1 PTPase; a dual specificity phosphatase. Receptor PTPases. Structurally,

all known receptor PTPases, are made up of a variable length extracellular domain, followed by a transmembrane region and a C-terminal catalytic cytoplasmic domain. Some of the receptor PTPases contain fibronectin type III (FN-III) repeats, immunoglobulin-like domains, MAM domains or carbonic anhydrase-like domains in their extracellular region. The cytoplasmic region generally contains two copies of the PTPase domain. The first seems to have enzymatic activity, while the second is inactive but seems to affect substrate specificity of the first. In these domains, the catalytic cysteine is generally conserved but some other, presumably important, residues are not. In the following table, the domain structure of known receptor PTPases is shown:

	Extracellular	Intracellular	
Ig FN-3	0 2 0 0 2	0 2 0 0 2	Leukocyte antigen related (LAR)
CAH MAM PTPase	0 1 0 0 1	0 1 0 0 1	Drosophila DLAR
Leukocyte antigen	0 0 0 0 0	0 0 0 0 0	Drosophila DPTP
related (LAR)	0 0 0 0 0	0 0 0 0 0	2 PTP-alpha
	0 0 0 0 0	0 0 0 0 0	2 PTP-beta
	0 0 0 0 0	0 0 0 0 0	1 PTP-gamma
	0 0 0 0 0	0 0 0 0 0	1 PTP-delta
	0 0 0 0 0	0 0 0 0 0	2 PTP-epsilon
	0 0 0 0 0	0 0 0 0 0	2 PTP-kappa
	0 0 0 0 0	0 0 0 0 0	1 PTP-mu
	0 0 0 0 0	0 0 0 0 0	1 PTP-zeta

PTPase domains consist of about 300 amino acids. There are two conserved cysteines, the second one has been shown to be absolutely required for activity. Furthermore, a number of conserved residues in its immediate vicinity have also been shown to be important. A signature pattern for PTPase domains was derived centered on the active site cysteine. There are three profiles for PTPases, the first one spans the complete domain and is not specific to any subtype. The second profile is specific to dual-specificity PTPases and the third one to the PTP subfamily

Consensus pattern: [LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAGP]-x-[LIVMFY] [C is the active site residue]-

- [1] Fischer E.H., Charbonneau H., Tonks N.K. Science 253:401-406(1991).
- [2] Charbonneau H., Tonks N.K. Annu. Rev. Cell Biol. 8:463-493(1992).
- [3] Trowbridge I.S. J. Biol. Chem. 266:23517-23520(1991).
- [4] Tonks N.K., Charbonneau H. Trends Biochem. Sci. 14:497-500(1989).
- [5] Hunter T. Cell 58:1013-1016(1989).

142. (DUF10) Uncharacterized protein family UPF0076 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Goat antigen UK114, a human homolog and the rat corresponding protein which is known as perchloric acid soluble protein (PSP1). PSP1 [2] may inhibit an initiation stage of cell-free

protein synthesis. - Mouse heat-responsive protein HRSP12. - Yeast chromosome V hypothetical protein YER057c. - Yeast chromosome IX hypothetical protein YIL051c. - *Caenorhabditis elegans* hypothetical protein C23G10.2. - *Escherichia coli* hypothetical protein ycdK. - *Escherichia coli* hypothetical protein yhaR. - *Escherichia coli* hypothetical protein yjgF and HI0719, the corresponding *Haemophilus influenzae* protein. - *Escherichia coli* hypothetical protein yoaB. - *Bacillus subtilis* hypothetical protein yabJ. - *Haemophilus influenzae* hypothetical protein HI1627. - *Helicobacter pylori* hypothetical protein HP0944. - *Lactococcus lactis* aldR. - *Myxococcus xanthus* dfrA. - *Synechocystis* strain PCC 6803 hypothetical protein slr0709. - *Rhizobium* strain NGR234 symbiotic plasmid hypothetical protein y4sK. - *Pyrococcus horikoshii* hypothetical protein PH0854. These are small proteins of around 15 Kd whose sequence is highly conserved. As a signature pattern, a well conserved region located in the C-terminal part of these proteins was selected.

Consensus pattern: [PA]-[ASTPV]-R-[SACVF]-x-[LIVMFY]-x(2)-[GSAKR]-x-[LMVA]-x(5,8)-[LIVM]-E-[MI]-

[1] Bairoch A. Unpublished observations (1995).

[2] Oka T., Tsuji H., Noda C., Sakai K., Hong Y.-M., Suzuki I., Munoz S., Natori Y. J. Biol. Chem. 270:30060-30067(1995).

143. (DUF3) Domain of Unknown Function 3

Domain apparently occurring exclusively in eubacteria. Unknown function.

144. (DUF6) Integral membrane protein

This family includes many hypothetical membrane proteins of unknown function.

Many of the proteins contain two copies of the aligned region.

145. (DUF7) Integral membrane protein

This family includes many hypothetical membrane proteins of unknown function.

Swiss:P14502 has been implicated in resistance to ethidium bromide.

146. (DapB) Dihydrodipicolinate reductase signature

Dihydrodipicolinate reductase (EC 1.3.1.26) catalyzes the second step in the biosynthesis of diaminopimelic acid and lysine, the NAD or NADP-dependent reduction of 2,3-dihydrodipicolinate into 2,3,4,5-tetrahydrodipicolinate. This enzyme is present in bacteria (gene *dapB*) and higher plants. As a signature pattern the best conserved region in this enzyme was selected. It is located in the central section and is part of the substrate-binding region [1].

Consensus pattern: E-[IV]-x-E-x-H-x(3)-K-x-D-x-P-S-G-T-A-

[1] Scapin G., Blanchard J.S., Sacchettini J.C. Biochemistry 34:3502-3512(1995).

147. DedA family

This family combines the DedA related proteins and YIAN/YGIK family. Members of this family are not functionally characterised. These proteins contain multiple predicted transmembrane regions.

148. DegT/DnrJ/EryC1/StrS family

The members of this family exhibit some characteristics of the sensor protein of two-component signal transduction systems, however none of the members show any sequence similarity to these protein kinases. The members of this family do have the typical helix-turn-helix motif of DNA binding proteins.

[1] Stutzman-Engwall KJ, Otten SL, Hutchinson CR, J Bacteriol 1992;174:144-154.

149. (Desaturase) Fatty acid desaturases signatures

Fatty acid desaturases (EC 1.14.99.-) are enzymes that catalyze the insertion of a double bond at the delta position of fatty acids. There seems to be two distinct families of fatty acid desaturases which do not seem to be evolutionary related. Family 1 is composed of: - Stearoyl-CoA desaturase (SCD) (EC 1.14.99.5) [1]. SCD is a key regulatory enzyme of

unsaturated fatty acid biosynthesis. SCD introduces a cis double bond at the delta(9) position of fatty acyl-CoA's such as palmitoleoyl- and oleoyl-CoA. SCD is a membrane-bound enzyme that is thought to function as a part of a multienzyme complex in the endoplasmic reticulum of vertebrates and fungi. As a signature pattern for this family a conserved region in the C-terminal part of these enzymes was selected, this region is rich in histidine residues and in aromatic residues. Family 2 is composed of: - Plants stearoyl-acyl-carrier-protein desaturase (EC 1.14.99.6) [2], these enzymes catalyze the introduction of a double bond at the delta(9) position of stearoyl-ACP to produce oleoyl-ACP. This enzyme is responsible for the conversion of saturated fatty acids to unsaturated fatty acids in the synthesis of vegetable oils. - Cyanobacteria desA [3] an enzyme that can introduce a second cis double bond at the delta(12) position of fatty acid bound to membranes glycerolipids. DesA is involved in chilling tolerance; the phase transition temperature of lipids of cellular membranes being dependent on the degree of unsaturation of fatty acids of the membrane lipids. As a signature pattern for this family a conserved region in the C-terminal part of these enzymes was selected.

Consensus pattern: G-E-x-[FY]-H-N-[FY]-H-H-x-F-P-x-D-Y-

Consensus pattern: [ST]-[SA]-x(3)-[QR]-[LI]-x(5,6)-D-Y-x(2)-[LIVMFYW]-[LIVM]-[DE]-

[1] Kaestner K.H., Ntambi J.M., Kelly T.J. Jr., Lane M.D. J. Biol. Chem. 264:14755-14761(1989).

[2] Shanklin J., Somerville C.R. Proc. Natl. Acad. Sci. U.S.A. 88:2510-2514(1991).

[3] Wada H., Gombos Z., Murata N. Nature 347:200-203(1990).

150. Dihydroorotase signatures

Dihydroorotase (EC 3.5.2.3) (DHOase) catalyzes the third step in the de novo biosynthesis of pyrimidine, the conversion of ureidosuccinic acid (N-carbamoyl-L-aspartate) into dihydroorotate. Dihydroorotase binds a zinc ion which is required for its catalytic activity [1].

In bacteria, DHOase is a dimer of identical chains of about 400 amino-acid residues (gene pyrC). In higher eukaryotes, DHOase is part of a large multi-functional protein known as 'rudimentary' in Drosophila and CAD in mammals and which catalyzes the first three steps of pyrimidine biosynthesis [2]. The DHOase domain is located in the central part of this polyprotein. In yeasts, DHOase is encoded by a monofunctional protein (gene URA4).

However, a defective DHOase domain [3] is found in a multifunctional protein (gene URA2) that catalyzes the first two steps of pyrimidine biosynthesis. The comparison of DHOase sequences from various sources shows [4] that there are two highly conserved regions. The first located in the N-terminal extremity contains two histidine residues suggested [3] to be involved in binding the zinc ion. The second is found in the C-terminal part. Signature patterns for both regions have been developed. Allantoinase (EC 3.5.2.5) is the enzyme that hydrolyzes allantoin into allantoate. In yeast (gene DAL1) [5], it is the first enzyme in the allantoin degradation pathway; in amphibians [6] and fish it catalyzes the second step in the degradation of uric acid. The sequence of allantoinase is evolutionary related to that of DHOases.

Consensus pattern: D-[LIVMFYWSAP]-H-[LIVA]-H-[LIVF]-[RN]-x-[PGANF] [The two H's are probable zinc ligands]-

Consensus pattern: [GA]-[ST]-D-x-A-P-H-x(4)-K-

[1] Brown D.C., Collins K.D. J. Biol. Chem. 266:1597-1604(1991).

[2] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B. BioEssays 15:157-164(1993).

[3] Souciet J.-L., Nagy M., Le Gouar M., Lacroute F., Potier S. Gene 79:59-70(1989).

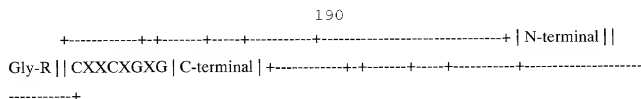
[4] Guyonvarch A., Nguyen-Juilleret M., Hubert J.-C., Lacroute F. Mol. Gen. Genet. 212:134-141(1988).

[5] Buckholz R.G., Cooper T.G. Yeast 7:913-923(1991).

[6] Hayashi S., Jain S., Chu R., Alvares K., Xu B., Erfurth F., Usuda N., Rao M.S., Reddy S.K., Noguchi T., Reddy J.K., Yeldandi A.Y. J. Biol. Chem. 269:12269-12276(1994).

151. dnaJ domains signatures and profile

The prokaryotic heat shock protein dnaJ interacts with the chaperone hsp70-like dnaK protein [1]. Structurally, the dnaJ protein consists of an N- terminal conserved domain (called 'J' domain) of about 70 amino acids, a glycine-rich region ('G' domain') of about 30 residues, a central domain containing four repeats of a CXXCXGXG motif ('CRR' domain) and a C-terminal region of 120 to 170 residues. Such a structure is shown in the following schematic representation:



It has been shown [2] that the 'J' domain as well as the 'CRR' domain are also found in other prokaryotic and eukaryotic proteins which are listed below.

a) Proteins containing both a 'J' and a 'CRR' domain:

- Yeast protein MAS5/YDJ1 which seems to be involved in mitochondrial protein import.
- Yeast protein MDJ1, involved in mitochondrial biogenesis and protein folding.
- Yeast protein SCJ1, involved in protein sorting.
- Yeast protein XDJ1.
- Plants dnaJ homologs (from leek and cucumber).
- Human HDJ2, a dnaJ homolog of unknown function.
- Yeast hypothetical protein YNL077w.

b) Proteins containing a 'J' domain without a 'CRR' domain:

- Rhizobium fredii nolC, a protein involved in cultivar-specific nodulation of soybean.
- Escherichia coli cbpA [3], a protein that binds curved DNA.
- Yeast protein SEC63/NPL1, important for protein assembly into the endoplasmic reticulum and the nucleus.
- Yeast protein SIS1, required for nuclear migration during mitosis.
- Yeast protein CAJ1.
- Yeast hypothetical protein YFR041c.
- Yeast hypothetical protein YIR004w.
- Yeast hypothetical protein YJL162c.
- Plasmodium falciparum ring-infected erythrocyte surface antigen (RESA). RESA, whose function is not known, is associated with the membrane skeleton of newly invaded erythrocytes.
- Human HDJ1.
- Human HSJ1, a neuronal protein.
- Drosophila cysteine-string protein (csp).

A signature pattern for the 'J' domain was developed, based on conserved positions in the C-terminal half of this domain. A pattern for the 'CRR' domain, based on the first two copies of that motif was also developed. A profile for the 'J' domain was also developed.

Consensus pattern: [FY]-x(2)-[LIVMA]-x(3)-[FYWHNT]-[DENQSA]-x-L-x-[DN]-x(3)-
[KR]-x(2)-[FYI]-

Consensus pattern: C-[DEGSTHKR]-x-C-x-G-x-[GK]-[AGSDM]-x(2)-[GSNKR]-x(4,6)-C-
x(2,3)-C-x-G-x-G-

[1] Cyr D.M., Langer T., Douglas M.G. Trends Biochem. Sci. 19:176-181(1994).

[2] Bork P., Sander C., Valencia A., Bukau B. Trends Biochem. Sci. 17:129-129(1992).

[3] Ueguchi C., Kaneda M., Yamada H., Mizuno T. Proc. Natl. Acad. Sci. U.S.A. 91:1054-

1058(1994).

152.

153. Dwarfins

This family known as the dwarfins also includes the drosophila protein MAD. The N-terminus of MAD can bind to DNA [2].

[1] Yingling JM, Das P, Savage C, Zhang M, Padgett RW, Wang XF, Proc Natl Acad Sci U S A 1996;93:8940-8944. [2] Kim J, Johnson K, Chen HJ, Carroll S, Laughon A, Nature 1997;388:304-308.

154. Dynein light chain type 1 signature

Dynein is a multisubunit microtubule-dependent motor enzyme that acts as the force generating protein of eukaryotic cilia and flagella. The cytoplasmic isoform of dynein acts as a motor for the intracellular retrograde motility of vesicles and organelles along microtubules. Dynein is composed of a number of ATP-binding large subunits, intermediate size subunits and small subunits. Among the small subunits, there is a family [1,2] of highly conserved proteins which consist of: - Chlamydomonas reinhardtii flagellar outer arm dynein 8 Kd and 11 Kd light chains. - Higher eukaryotes cytoplasmic dynein light chain 1. - Yeast cytoplasmic dynein light chain 1 (gene DYN2 or SLC1). - Caenorhabditis elegans hypothetical dynein light chains M18.2 and T26A5.9. These proteins have from 89 to 120 amino acids. As a signature pattern, A highly conserved region was selected.

Consensus pattern: H-x-I-x-G-[KR]-x-F-[GA]-S-x-V-[ST]-[HY]-E -

[1] King S.M., Patel-King R.S. J. Biol. Chem. 270:11445-11452(1995).

[2] Dick T., Ray K., Salz H.K., Chia W. Mol. Cell. Biol. 16:1966-1977(1996).

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155. dUTPase

dUTPase hydrolyzes dUTP to dUMP and pyrophosphate.

- [1] Cedergren-Zeppezauer ES, Larsson G, Nyman PO, Dauter Z, Wilson KS, Nature 1992;355:740-743. [2] Mol CD, Harris JM, McIntosh EM, Tainer JA, Structure 1996;4:1077-1092.

156. (dCMP cyt deam) Cytidine and deoxycytidylate deaminases zinc-binding region

15 signature

- Cytidine deaminase (EC 3.5.4.5) (cytidine aminohydrolase) catalyzes the hydrolysis of cytidine into uridine and ammonia while deoxycytidylatedeaminase (EC 3.5.4.12) (dCMP deaminase) hydrolyzes dCMP into dUMP. Both enzymes are known to bind zinc and to require it for their catalytic activity[1,2]. These two enzymes do not share any sequence similarity with the exception of a region that contains three conserved histidine and cysteine residues which are thought to be involved in the binding of the catalytic zincion. Such a region is also found in other proteins [3,4]: - Yeast cytosine deaminase (EC 3.5.4.1) (gene FCY1) which transforms cytosine into uracil. - Mammalian apolipoprotein B mRNA editing protein, responsible for the postranscriptional editing of a CAA codon into a UAA (stop) codon in the APOB mRNA. - Riboflavin biosynthesis protein ribG, which converts 2,5-diamino-6- (ribosylamino)-4(3H)-pyrimidinone 5'-phosphate into 5-amino-6- (ribosylamino)-2,4(1H,3H)-pyrimidinedione 5'-phosphate. - *Bacillus cereus* blasticidin-S deaminase (EC 3.5.4.23), which catalyzes the deamination of the cytosine moiety of the antibiotics blasticidin S, cytomycin and acetylblasticidin S. - *Bacillus subtilis* protein comEB. This protein is required for the binding and uptake of transforming DNA. - *Bacillus subtilis* hypothetical protein yaaJ. - *Escherichia coli* hypothetical protein yfhC. - Yeast hypothetical protein YJL035c. A signature pattern for this zinc-binding region was derived.

Consensus pattern: [CH]-[AGV]-E-x(2)-[LIVMFGAT]-[LIVM]-x(17,33)-P-C-x(2,8)-C-x(3)-[LIVM] [The C's and H are zinc ligands]

- [1] Yang C., Carlow D., Wolfenden R., Short S.A. Biochemistry 31:4168-4174(1992).
 5 [2] Moore J.T., Silversmith R.E., Maley G.F., Maley F. J. Biol. Chem. 268:2288-2291(1993).
 [3] Reizer J., Buskirk S., Bairoch A., Reizer A., Saier M.H. Jr. Protein Sci. 3:853-856(1994).
 [4] Bhattacharya S., Navaratnam N., Morrison J.R., Scott J., Taylow W.R. Trends Biochem. Sci. 19:105-106(1994).

157. Dehydrins signatures

A number of proteins are produced by plants that experience water-stress. Water-stress takes place when the water available to a plant falls below a critical level. The plant hormone abscisic acid (ABA) appears to modulate the response of plant to water-stress. Proteins that are expressed during water-stress are called dehydrins [1,2] or LEA group 2 proteins [3]. The proteins that belong to this family are listed below.

- Arabidopsis thaliana XERO 1, XERO 2 (LTI30), RAB18, ERD10 (LTI45) ERD14 and COR47.
- Barley dehydrins B8, B9, B17, and B18.
- Cotton LEA protein D-11.
- Craterostigma plantagineum dessication-related proteins A and B.
- Maize dehydrin M3 (RAB-17).
- Pea dehydrins DHN1, DHN2, and DHN3.
- Radish LEA protein.
- Rice proteins RAB 16B, 16C, 16D, RAB21, and RAB25.
- Tomato TAS14.
- Wheat dehydrin RAB 15 and cold-shock protein cor410, cs66 and cs120.

Dehydrins share a number of structural features. One of the most notable features is the presence, in their central region, of a continuous run of five to nine serines followed by a cluster of charged residues. Such a region has been found in all known dehydrins so far with the exception of pea dehydrins. A second conserved feature is the presence of two copies of lysine-rich octapeptide: the first copy is located just after the cluster of charged residues that follows the poly-serine region and the second copy is found at the C-terminal extremity. Signature patterns for both regions were derived.

Consensus pattern: S(5)-[DE]-x-[DE]-G-x(1,2)-G-x(0,1)-[KR](4

Consensus pattern: [KR]-[LIM]-K-[DE]-K-[LIM]-P-G-

[1] Close T.J., Kortt A.A., Chandler P.M. *Plant Mol. Biol.* 13:95-108(1989).

5 [2] Robertson M., Chandler P.M. *Plant Mol. Biol.* 19:1031-1044(1992).

[3] Dure L. III, Crouch M., Harada J., Ho T.-H. D., Mundy J., Quatrano R., Thomas T., Sung Z.R. *Plant Mol. Biol.* 12:475-486(1989).

10 158. (deoR) Bacterial regulatory proteins, deoR family signature

The many bacterial transcription regulation proteins which bind DNA through a helix-turn-helix' motif can be classified into subfamilies on the basis of sequence similarities. One of these subfamilies groups the following proteins[1,2]: - accR, the *Agrobacterium tumefaciens* plasmid pTiC58 repressor of opine catabolism and conjugal transfer. - agaR, the *Escherichia coli* aga operon putative repressor. - deoR, the *Escherichia coli* deoxyribose operon repressor. - fucR, the *Escherichia coli* L-fucose operon activator. - gatR, the *Escherichia coli* galactitol operon repressor. - glpR, the *Escherichia coli* glycerol-3-phosphate regulon repressor. - gutR (or srlR), the *Escherichia coli* glucitol operon repressor. - iolR, from *Bacillus subtilis*. - lacR, the *Streptococcus lactose* phosphotransferase system repressor. - spoIIID, the *Bacillus subtilis* transcription regulator of the sigK gene. - yfjR, an *Escherichia coli* hypothetical protein. - ygbI, an *Escherichia coli* hypothetical protein. - yihW, an *Escherichia coli* hypothetical protein. - yjfQ, an *Escherichia coli* hypothetical protein. - yjhJ, an *Escherichia coli* hypothetical protein. The 'helix-turn-helix' DNA-binding motif of these proteins is located in the N-terminal part of the sequence. The pattern used to detect these proteins starts fourteen residues before the HTH motif and ends one residue after it.

Consensus pattern: R-x(3)-[LIVM]-x(3)-[LIVM]-x(16,17)-[STA]-x(2)-T-[LIVMA]-[RH]-[KRNA]-D-[LIVMF]-

30 [1] von Bodman S., Hayman G.T., Farrand S.K. *Proc. Natl. Acad. Sci. U.S.A.* 89:643-647(1992).

[2] Bairoch A. Unpublished observations (1993).

159. dsrm

Double-stranded RNA binding motif

[1] Burd CG, Dreyfuss G; Medline: 94310455, Conserved structures and diversity of functions of RNA-binding proteins. Science 1994;265:615-621.

Sequences gathered for seed by HMM_iterative_training Putative motif shared by proteins that bind to dsRNA. At least some DSRM proteins seem to bind to specific RNA targets. Exemplified by Staufen, which is involved in localization of at least five different mRNAs in the early Drosophila embryo. Also by interferon-induced protein kinase in humans, which is part of the cellular response to dsRNA.

Number of members: 116

160. Dynamin family signature

Dynamin [1,2] is a microtubule-associated force-producing protein of 100 Kd which is involved in the production of microtubule bundles and which is able to bind and hydrolyze GTP. Dynamin is structurally related to the following proteins: - Drosophila shibire protein (gene shi) [3]. Shibire is, very probably, the Drosophila cognate of mammalian dynamin. It seems to provide the motor for vesicular transport during endocytosis. - Yeast vacuolar sorting protein VPS1 (or SPO15) [4], a protein which could also be involved in microtubule-associated motility. - Yeast protein MGM1 [5], which is required for mitochondrial genome maintenance. - Yeast protein DNM1, which is involved in endocytosis. - Interferon induced Mx proteins [6,7]. Interferon alpha or beta induce the synthesis of a family of closely related proteins. Most of these proteins are known to confer resistance to influenza viruses and/or rhabdoviruses on transfected mammalian cell in culture. The three motifs found in all GTP-binding proteins are located in the N-terminal part of these proteins. The signature pattern that was developed for these proteins is based on a highly conserved region downstream of the ATP/GTP-binding motif 'A' (P-loop) (see <PDOC00017>).-

Consensus pattern: L-P-[RK]-G-[STN]-[GN]-[LIVM]-V-T-R-

[1] Vallee R.B., Shpetner H.S. Annu. Rev. Biochem. 59:909-932(1990).

[2] Obar R.A., Collins C.A., Hammarback J.A., Shpetner H.S., Vallee R.B. *Nature* 347:256-261(1990).

[3] van der Blik A., Meyerowitz E.M. *Nature* 351:411-414(1991).

[4] Rothman J.H., Raymond C.K., Gilbert T., O'Hara P.J., Stevens T.H. Cell 61:1063-1074(1990).

[5] Jones B.A., Fangman W.L. *Genes Dev.* 6:380-389(1992).

[6] Arnheiter H., Meier E. *New Biol.* 2:851-857(1990).

[7] Staeheli P., Pitossi F., Pavlovic J. *Trends Cell Biol.* 3:268-272(1993).

161. (dynamine_2) Dynamine central region

This region lies between the GTPase domain, see dynamine, and the pleckstrin homology (PH) domain.

162. E1-E2 ATPases phosphorylation site

E1-E2 ATPases (also known as P-type) are cation transport ATPases which form an aspartyl phosphate intermediate in the course of ATP hydrolysis. ATPases which belong to this family are listed below [1,2,3]. - Fungal and plant plasma membrane (H⁺) ATPases [reviewed in 4].

- Vertebrate (Na⁺, K⁺) ATPases (sodium pump) [reviewed in 5,6]. - Gastric (K⁺, H⁺) ATPases (proton pump). - Calcium (Ca⁺⁺) ATPases (calcium pump) from the sarcoplasmic reticulum (SR), the endoplasmic reticulum (ER) and the plasma membrane. - Copper (Cu⁺⁺) ATPases (copper pump) which are involved in two human genetic disorders: Menkes syndrome and Wilson disease [7]. - Bacterial potassium (K⁺) ATPases. - Bacterial cadmium efflux (Cd⁺⁺) ATPases [reviewed in 8]. - Bacterial magnesium (Mg⁺⁺) ATPases. - A probable cation ATPase from *Leishmania*. - fixL, a probable cation ATPase from *Rhizobium meliloti*, involved in nitrogen fixation. The region around the phosphorylated aspartate residue is perfectly conserved in all these ATPases and can be used as a signature pattern.

Consensus pattern: D-K-T-G-T-[LI]-[TI] [D is phosphorylated]

[1] Green N.M., McLennan D.H. *Biochem. Soc. Trans.* 17:819-822(1989).

[2] Green N.M. *Biochem. Soc. Trans.* 17:970-972(1989).

[3] Fagan M.J., Saier M.H. Jr. *J. Mol. Evol.* 38:57-99(1994).

- [4] Serrano R. Biochim. Biophys. Acta 947:1-28(1988).
- [5] Fambrough D.M. Trends Neurosci. 11:325-328(1988).
- [6] Sweadner K.J. Biochim. Biophys. Acta 988:185-220(1989).
- [7] Bull P.C., Cox D.W. Trends Genet. 10:246-251(1994).
- 5 [8] Silver S., Nucifora G., Chu L., Misra T.K. Trends Biochem. Sci. 14:76-80(1989).

163. E1_N

E1 Protein, N terminal domain

10 Number of members: 90

164. (E1_dehydrog) Dehydrogenase E1 component

This family uses thiamine pyrophosphate as a cofactor. This family includes pyruvate

15 dehydrogenase, 2-oxoglutarate dehydrogenase and 2-oxoisovalerate dehydrogenase.

165. (ECH) Enoyl-CoA hydratase/isomerase signature

Enoyl-CoA hydratase (EC 4.2.1.17) (ECH) [1] and 3-2trans-enoyl-CoA isomerase(EC
20 5.3.3.8) (ECI) [2] are two enzymes involved in fatty acid metabolism. ECH catalyzes the
hydration of 2-trans-enoyl-CoA into 3-hydroxyacyl-CoA and ECI shifts the 3- double bond
of the intermediates of unsaturated fatty acid oxidation to the 2-trans position. Most
eukaryotic cells have two fatty-acid beta-oxidation systems, one located in mitochondria and
the other in peroxisomes. In mitochondria, ECH and ECI are separate yet structurally related
25 monofunctional enzymes. Peroxisomes contain a trifunctional enzyme [3] consisting of an N-
terminal domain that bears both ECH and ECI activity, and a C-terminal domain responsible
for 3-hydroxyacyl-CoA dehydrogenase (HCDH) activity. In Escherichia coli (gene fadB) and
Pseudomonas fragi (gene faoA), ECH and ECI are also part of a multifunctional enzyme
which contains both a HCDH and a3-hydroxybutyryl-CoA epimerase domain [4].A number
30 of other proteins have been found to be evolutionary related to the ECH/ECI enzymes or
domains: - 3-hydroxybutyryl-coa dehydratase (EC 4.2.1.55) (crotonase), a bacterial enzyme
involved in the butyrate/butanol-producing pathway. - Naphthoate synthase (EC 4.1.3.36)
(DHNA synthetase) (gene menB) [5], a bacterial enzyme involved in the biosynthesis of
menaquinone (vitamin K2). DHNA synthetase converts O-succinyl-benzoyl-CoA (OSB-

CoA) to 1,4-dihydroxy- 2-naphthoic acid (DHNA). - 4-chlorobenzoate dehalogenase (EC 3.8.1.6) [6], a *Pseudomonas* enzyme which catalyzes the conversion of 4-chlorobenzoate-CoA to 4-hydroxybenzoate-CoA. - A *Rhodobacter capsulatus* protein of unknown function (ORF257) [7]. - *Bacillus subtilis* putative polyketide biosynthesis proteins pksH and pksI. - 5 *Escherichia coli* carnitine racemase (gene caiD) [8]. - *Escherichia coli* hypothetical protein ygfG. - Yeast hypothetical protein YDR036c. As a signature pattern for these enzymes, a conserved region rich in glycine and hydrophobic residues was selected.

Consensus pattern: [LIVM]-[STA]-x-[LIVM]-[DENQRHSTA]-G-x(3)-[AG](3)-x(4)-

10 [LIVMST]-x-[CSTA]-[DQHP]-[LIVMFY]-

[1] Minami-Ishii N., Taketani S., Osumi T., Hashimoto T. Eur. J. Biochem. 185:73-78(1989).

[2] Mueller-Newen G., Stoffel W. Biol. Chem. Hoppe-Seyler 372:613-624(1991).

15 [3] Palosaari P.M., Hiltunen J.K. J. Biol. Chem. 265:2446-2449(1990).

[4] Nakahigashi K., Inokuchi H. Nucleic Acids Res. 18:4937-4937(1990).

[5] Driscoll J.R., Taber H.W. J. Bacteriol. 174:5063-5071(1992).

[6] Babbitt P.C., Kenyon G.L., Matin B.M., Charest H., Sylvestre M., Scholten J.D., Chang K.-H., Liang P.-H., Dunaway-Mariano D. Biochemistry 31:5594-5604(1992).

20 [7] Beckman D.L., Kranz R.G. Gene 107:171-172(1991).

[8] Eichler K., Bourgis F., Buchet A., Kleber H.-P., Mandrand-Berthelot M.-A. Mol. Microbiol. 13:775-786(1994).

25 166. (EF1BD) Elongation factor 1 beta/beta'/delta chain signatures

Eukaryotic elongation factor 1 (EF-1) is responsible for the GTP-dependent binding of aminoacyl-tRNAs to the ribosomes [1]. EF-1 is composed of four subunits: the alpha chain which binds GTP and aminoacyl-tRNAs, the gamma chain that probably plays a role in anchoring the complex to other cellular components and the beta and delta (or beta') chains.

30 The beta and delta chains are highly similar proteins that both stimulate the exchange of GDP bound to the alpha chain for GTP [2]. The beta and delta chains are hydrophilic proteins of around 23 to 31 Kd. Their C-terminal part seems important for the nucleotide exchange activity, while the N-terminal section is probably involved in the interaction with the gamma chain. Two signature patterns for this family of proteins were developed. The first

corresponds to an acidic region in the central section; the second, to the C-terminal extremity of these proteins

Consensus pattern: [DE]-[DEG]-[DE](2)-[LIVMF]-D-L-F-G-

Consensus pattern: [IV]-O-S-x-D-[LIVM]-x-A-[FWM]-[NQ]-K-[LIVM]-

[1] Riis B., Rattan I.S., Clark B.F.C., Merrick W.C. Trends Biochem. Sci. 15:420-424(1990).

[2] van Damme H.T.F., Amons R., Karssies R., Timmers C.J., Janssen G.M.C., Moeller W. Biochim. Biophys. Acta 1050:241-247(1990).

167. (EF1G_domain) Elongation factor 1 gamma, conserved domain

168. (EFG_C) Elongation factor G C-terminus

This family is always found associated with GTP_EFTU. This family includes the carboxyl terminal regions of Elongation factor G, elongation factor 2 and some tetracycline resistance proteins.

169. (EFP) Elongation factor P signature

Elongation factor P (EF-P) [1] is a prokaryotic protein translation factor required for efficient peptide bond synthesis on 70S ribosomes from fMet-tRNA^{fMet}. EF-P is a protein of 21 Kd.

It is evolutionary related to yeiP, an hypothetical protein from Escherichia coli. As a

signature pattern, a conserved region located in the C-terminal part of these proteins was selected.

Consensus pattern: K-x-[AV]-x(4)-G-x(2)-[LIV]-x-V-P-x(2)-[LIV]-x(2)-G-

[1] Aoki H., Adams S.-L., Turner M.A., Ganoza M.C. Biochimie 79:7-11(1997).

170. (EF TS) Elongation factor Ts signatures

In prokaryotes elongation factor Ts (EF-Ts) is a component of the elongation cycle of protein biosynthesis. It associates with the EF-Tu.GDP complex and induces the exchange of GDP to GTP, it remains bound to the aminoacyl-tRNA.EF-Tu.GTP complex up to the GTP hydrolysis stage on the ribosome [1].EF-Ts is also a component of the chloroplast protein biosynthetic machinery and is encoded in the genome of some algal chloroplast [2]. It is also present in mitochondria [3]. As signature patterns for EF-Ts, two conserved regions located in the N-terminal part of the protein have been selected.

Consensus pattern: L-R-x(2)-T-[GSDNQ]-x-[GS]-[LIVMF]-x(0,1)-[DENKAC]-x-K-

[KRNEQS]-A-L-

Consensus pattern: E-[LIVM]-[NV]-[SCV]-[QE]-T-D-F-V-[SA]-[KRN]-

[1] Bubunenko M.G., Kireeva M.L., Gudkov A.T. *Biochimie* 74:419-425(1992).

[2] Kostrzewa M., Zetsche K. *Plant Mol. Biol.* 23:67-76(1993).

[3] Xin H., Worlax V.L., Burkhardt W.A., Spremulli L.L. *J. Biol. Chem.* 270:17243-17249(1995).

171. (EMP24_GP25L) emp24/gp25L/p24 family

Members of this family are implicated in bringing cargo forward from the ER and binding to coat proteins by their cytoplasmic domains. Number of members: 30

Paccaud JP, Thomas DY, Bergeron JJ, Nilsson T, *J Cell Biol* 1998;140:751-765.

172. ENV_polypotein

ENV polypotein (coat polypotein)

Number of members: 224

173. (ERG4_ERG24) Ergosterol biosynthesis ERG4/ERG24 family signatures

Two fungal enzymes involved in ergosterol biosynthesis and which act by reducing double bonds in precursors of ergosterol have been shown to be evolutionary related [1]. These are C-14 sterol reductase (gene ERG24 in budding yeast and erg3 in *Neurospora Crassa*) and C-24(28) sterol reductase (gene ERG4 in budding yeast and sts1 in fission yeast). Their

sequences are also highly related to that of chicken lamin B receptor, which is thought to anchor the lamina to the inner nuclear membrane. These proteins are highly hydrophobic and seem to contain seven or eight transmembrane regions. As signature patterns, two conserved regions were selected. The first one is apparently located in a loop between the fourth and fifth transmembrane regions and the second is in the C-terminal section.

Consensus pattern: G-x(2)-[LIVM]-[YH]-D-x-[FYW]-x-G-x(2)-L-N-P-R -

Consensus pattern: [LIVM](2)-H-R-x(2)-R-D-x(3)-C-x(2)-K-Y-G-

[1] Lai M.H., Bard M., Pierson C.A., Alexander J.F., Goebel M., Carter G.T., Kirsch D.R. Gene 140:41-49(1994).

174. (ERM) Ezrin/radixin/moesin family

This family of proteins contain a band 4.1 domain (Band 41), at their amino terminus. This family represents the rest of these proteins.

[1] Yonemura S, Hirao M, Doi Y, Takahashi N, Kondo T, Tsukita S, J Cell Biol 1998;140:885-895.

175. ER lumen protein retaining receptor signatures

Proteins that reside in the lumen of the endoplasmic reticulum (ER) contain a C-terminal tetrapeptide (generally K-D-E-L or H-D-E-L) that serves as a signal for their retrieval (retrograde transport) from subsequent compartments of the secretory pathway. The signal is recognized by a receptor molecule that is believed to cycle between the cis side of the Golgi apparatus and the ER [1]. This protein is known as the ER lumen protein retaining receptor or also as the 'KDEL receptor'. It has been characterized in a variety of species, including fungi (gene ERD2), plants, Plasmodium, Drosophila and mammals. In mammals two highly related forms of the receptor are known. Structurally, the receptor is a protein of about 220 residues that seems to contain seven transmembrane regions [2]. The N-terminal part (3 residues) is oriented toward the lumen while the C-terminal tail (about 12 residues) is cytoplasmic. There are three luminal and three cytoplasmic loops. Two signature patterns for these receptors were developed. The first pattern corresponds to the C-terminal half of the first cytoplasmic loop as well as most of the second transmembrane domain. The second pattern is a perfectly

conserved decapeptide that corresponds to the central part of the fifth transmembrane domain.

Consensus pattern: G-I-S-x-[KR]-x-Q-x-L-[FY]-x-[LIV](2)-F-x(2)-R-Y-

5 Consensus pattern: L-E-[SA]-V-A-I-[LM]-P-Q-L-

[1] Pelham H.R.B. Curr. Opin. Cell Biol. 3:585-591(1991).

[2] Townsley F.M., Wilson D.W., Pelham H.R.B. EMBO J. 12:2821-2829(1993).

10

176. (ETF_beta) Electron transfer flavoprotein beta-subunit signature

The electron transfer flavoprotein (ETF) [1,2] serves as a specific electron acceptor for various mitochondrial dehydrogenases. ETF transfers electrons to the main respiratory chain via ETF-ubiquinone oxidoreductase. ETF is an heterodimer that consist of an alpha and a beta subunit and which bind one molecule of FAD per dimer. A similar system also exists in some bacteria. The beta subunit of ETF is a protein of about 28 Kd which is structurally related to the bacterial nitrogen fixation protein fixA which could play a role in a redox process and feed electrons to ferredoxin. Other related proteins are: - Escherichia coli hypothetical protein ydiQ. - Escherichia coli hypothetical protein ygcR. As a signature pattern
15 for these proteins, a conserved region which is located in the central section was selected.

Consensus pattern: [IVA]-x-[KR]-x(2)-[DE]-[GD]-[GDE]-x(1,2)-[EQ]-x-[LIV]- x(4)-P-x-[LIVM](2)-[TAC]-

25 [1] Finocchiaro G., Ikeda Y., Ito M., Tanaka K. Prog. Clin. Biol. Res. 321:637-652(1990).

[2] Tsai M.H., Saier M.H. Jr. Res. Microbiol. 146:397-404(1995).

177. Endonuclease III signatures

30 Escherichia coli endonuclease III (EC 4.2.99.18) (gene nth) [1] is a DNA repair enzyme that acts both as a DNA N-glycosylase, removing oxidized pyrimidines from DNA, and as an apurinic/apyrimidinic (AP) endonuclease, introducing a single-strand nick at the site from which the damaged base was removed. Endonuclease III is an iron-sulfur protein that binds a single 4Fe-4S cluster. The 4Fe-4S cluster does not seem to be important for catalytic activity,

- but is probably involved in the proper positioning of the enzyme along the DNA strand
- [2]. Endonuclease III is evolutionary related to the following proteins: - Fission yeast endonuclease III homolog (gene *nth1*) [3]. - *Escherichia coli* and related protein DNA repair protein *mutY*, which is an adenine glycosylase. *MutY* is a larger protein (350 amino acids)
- 5 than endonuclease III (211 amino acids). - *Micrococcus luteus* ultraviolet N-glycosylase/AP lyase which initiates repair at *cis-syn* pyrimidine dimers. - ORF10 in plasmid pFV1 of the thermophilic archaeobacteria *Methanobacterium thermoformicicum* [4]. Restriction methylase *m.MthTI*, which is encoded by this plasmid, generates 5-methylcytosine which is subject to deamination resulting in G-T mismatches. This protein could correct these mismatches. -
- 10 Yeast hypothetical protein YAL015c. - Fission yeast hypothetical protein SpAC26A3.02. - *Caenorhabditis elegans* hypothetical protein R10E4.5. - *Methanococcus jannaschii* hypothetical protein MJ0613. The 4Fe-4S cluster is bound by four cysteines which are all located in a 17 amino acid region at the C-terminal end of endonuclease III. A similar region is also present in the central section of *mutY* and in the C-terminus of ORF10 and of the
- 15 *Micrococcus UV* endonuclease. The 4Fe-4S cluster region does not exist in YAL015c. Two signature patterns for these proteins were developed: the first corresponds to the core of the iron-sulfur binding domain, the second corresponds to the best conserved region in the catalytic core of these enzymes.
- 20 Consensus pattern: C-x(3)-[KRS]-P-[KRAL]-C-x(2)-C-x(5)-C [The four C's are 4Fe-4S ligands]-
 Consensus pattern: [GST]-x-[LIVMF]-P-x(5)-[LIVMW]-x(2,3)-[LI]-[PAS]-G-V-[GA]-x(3)-
 [GAC]-x(3)-[LIVM]-x(2)-[SALV]-[LIVMFYW]-[GANK]-
- 25 [1] Kuo C.-F., McRee D., Fisher C.L., O'Handley S.F., Cunningham R.P., Tainer J.A. *Science* 258:434-440(1992).
 [2] Thomson A.J. *Curr. Biol.* 3:173-174(1993).
 [3] Roldan-Arjona T., Anselmino C., Lindahl T. *Nucleic Acids. Res.* 3307-3312(1996).
 [4] Noelling J., van Eeden F.J.M., Eggen R.I.L., de Vos W.M. *Nucleic Acids Res.* 20:6501-
 30 6507(1992).

178. (Epimerase) NAD dependent epimerase/dehydratase family

This family of proteins utilize NAD as a cofactor. The proteins in this family use nucleotide-sugar substrates for a variety of chemical reactions.

[1] Thoden JB, Hegeman AD, Wesenberg G, Chapeau MC, Frey PA, Holden HM, Biochemistry 1997;36:6294-6304.

5

179. Exonuclease

This family includes a variety of exonuclease proteins, such as ribonuclease T and the epsilon subunit of DNA polymerase III.

10

[1] Koonin EV, Deutscher MP, Nucleic Acids Res 1993;21:2521-2522.

180. ENTH

ENTH domain

15

[1] Kay BK, Yamabhai M, Wendland B, Emr SD; Medline: 99156083, Identification of a novel domain shared by putative components of the endocytic and cytoskeletal machinery. Protein Sci 1999;8:435-438.

20

The ENTH (Epsin N-terminal homology) domain is found in proteins involved in endocytosis and cytoskeletal machinery. The function of the ENTH domain is unknown.

Number of members: 29

25

181. (eIF-1A) Eukaryotic initiation factor 1A signature

Eukaryotic translation initiation factor 1A (eIF-1A) [1] (formerly known as eIF-4C) is a protein that seems to be required for maximal rate of protein biosynthesis. It enhances ribosome dissociation into subunits and stabilizes the binding of the initiator Met-tRNA to 40S ribosomal subunits. eIF-1A is a hydrophilic protein of about 15 to 17 Kd. Archaeobacteria also seem to possess a eIF-1A homolog. As a signature pattern, a conserved region in the central section of these proteins was selected.

30

Consensus pattern: [IM]-x-G-x-[GS]-[KRH]-x(4)-[CL]-x-D-G-x(2)-R-x(2)-[RH]-I- x-G

[1] Wei C.-L., Kainuma M., Hershey J.W.B. *J. Biol. Chem.* 270:22788-22794(1995).

5 182. (eIF-5A) Eukaryotic initiation factor 5A hypusine signature

Eukaryotic initiation factor 5A (eIF-5A) (formerly known as eIF-4D) [1,2] is a small protein whose precise role in the initiation of protein synthesis is not known. It appears to promote the formation of the first peptide bond. eIF-5A seems to be the only eukaryotic protein to contain an hypusine residue. Hypusine is derived from lysine by the post-translational
10 addition of a butylamino group (from spermidine) to the epsilon-amino group of lysine. The hypusine group is essential to the function of eIF-5A. A hypusine-containing protein has been found in archaeobacteria such as *Sulfolobus acidocaldarius* or *Methanococcus jannaschii*; this protein is highly similar to eIF-5A and could play a similar role in protein biosynthesis. The signature developed for eIF-5A is centered around the hypusine residue.

15 Consensus pattern: [PT]-G-K-H-G-x-A-K [The first K is modified to hypusine]

[1] Park M.H., Wolff E.C., Folk J.E. *Biofactors* 4:95-104(1993).

[2] Schnier J., Schwelberger H.G., Smit-McBride Z., Kang H.A., Hershey J.W.B. *Mol. Cell.*
20 *Biol.* 11:3105-3114(1991).

183. (efhand) S-100/ICaBP type calcium binding protein signature

S-100 are small dimeric acidic calcium and zinc-binding proteins [1] abundant in the brain.

25 They have two different types of calcium-binding sites: a low affinity one with a special structure and a 'normal' EF-hand type high affinity site. The vitamin-D dependent intestinal calcium-binding proteins (ICaBP or calbindin 9 Kd) also belong to this family of proteins, but it does not form dimers. In the past years the sequences of many new members of this family have been determined (for reviews see [2,3,4]); in most cases the function of these

30 proteins is not yet known, although it is becoming clear that they are involved in cell growth and differentiation, cell cycle regulation and metabolic control. These proteins are: -

- Calcyclin (Prolactin receptor associated protein (PRA); clatropin; 2a9; 5B10; S100A6). -

- Calpactin I light chain (p10; p11; 42c; S100A10). - Calgranulin A (cystic fibrosis antigen

(CFAg); MIF related protein 8 (MRP- 8); p8; S100A8). - Calgranulin B (MIF related protein

14 (MRP-14); p14; S100A9). - Calgranulin C. - Calgizzarin (S100C). - Placental calcium-binding protein (CAPL) (18a2; pL98; 42a; p9K; MTS1; metastatin; S100A4). - Protein S-100D (S100A5). - Protein S-100E (S100A3). - Protein S-100L (CAN19; S100A2). - Placental protein S-100P (S100E). - Psoriasin (S100A7). - Chemotactic cytokine CP-10 [5]. -

5 Protein MRP-126 [6]. - Trichohyalin [7]. This is a large intermediate filament-associated protein that associates with keratin intermediate filaments (KIF); it contains a S- 100 type domain in its N-terminal extremity. A number of these proteins are known to bind calcium while others are not (p10 for example). Our EF-hand detecting pattern will fail to pick those proteins which have lost their calcium-binding properties. A pattern was developed which

10 unambiguously picks up proteins belonging to this family. This pattern spans the region of the EF-hand high affinity site but makes no assumptions on the calcium-binding properties of this site.

Consensus pattern: [LIVMFYW](2)-x(2)-[LK]-D-x(3)-[DN]-x(3)-[DNSG]-[FY]-x- [ES]-
 15 [FYVC]-x(2)-[LIVMFS]-[LIVMF]

[1] Baudier J. (In) Calcium and Calcium Binding proteins, Gerday C., Bollis L., Giller R., Eds., pp102-113, Springer Verlag, Berlin, (1988).

[2] Moncrief N.D., Kretsinger R.H., Goodman M. J. Mol. Evol. 30:522-562(1990).

20 [3] Kligman D., Hilt D.C. Trends Biochem. Sci. 13:437-443(1988).

[4] Schaefer B.W., Wicki R., Engelkamp D., Mattei M.-G., Heizmann C.W. Genomics 25:638-643(1995).

[5] Lackmann M., Cornish C.J., Simpson R.J., Moritz R.L., Geczy C.L. J. Biol. Chem. 267:7499-7504(1992).

25 [6] Nakano T., Graf T. Oncogene 7:527-534(1992).

[7] Lee S.-C., Kim I.-G., Marekov L.N., O'Keefe E.J., Parry D.A.D., Steinert P.M., J. Biol. Chem. 268:12164-12176(1993).

EF-hand calcium-binding domain

30 Many calcium-binding proteins belong to the same evolutionary family and share a type of calcium-binding domain known as the EF-hand [1 to 5]. This type of domain consists of a twelve residue loop flanked on both side by a twelve residue alpha-helical domain. In an EF-hand loop the calcium ion is coordinated in a pentagonal bipyramidal configuration. The six residues

involved in the binding are in positions 1, 3, 5, 7, 9 and 12; these residues are denoted by X, Y, Z, -Y, -X and -Z. The invariant Glu or Asp at position 12 provides two oxygens for liganding Ca (bidentate ligand).

Listed below are the proteins which are known to contain EF-hand regions. For

- 5 each type of protein the total number of EF-hand regions known or supposed to exist is indicated between parenthesis. This number does not include regions which clearly have lost their calcium-binding properties, or the atypical low-affinity site (which spans thirteen residues) found in the S-100/ICaBP family of proteins [6].
- 10 - Aequorin and Renilla luciferin binding protein (LBP) (Ca=3).
- Alpha actinin (Ca=2). - Calbindin (Ca=4).
- Calcineurin B subunit (protein phosphatase 2B regulatory subunit) (Ca=4).
- Calcium-binding protein from *Streptomyces erythraeus* (Ca=3?).
- Calcium-binding protein from *Schistosoma mansoni* (Ca=2?).
- 15 - Calcium-binding proteins TCBP-23 and TCBP-25 from *Tetrahymena thermophila* (Ca=4?). - Calcium-dependent protein kinases (CDPK) from plants (Ca=4).
- Calcium vector protein from *amphoxius* (Ca=2).
- Calcyphosin (thyroid protein p24) (Ca=4?).
- Calmodulin (Ca=4, except in yeast where Ca=3).
- 20 - Calpain small and large chains (Ca=2). - Calretinin (Ca=6).
- Calcyclin (prolactin receptor associated protein) (Ca=2).
- Caltractin (centrin) (Ca=2 or 4).
- Cell Division Control protein 31 (gene CDC31) from yeast (Ca=2?).
- Diacylglycerol kinase (EC 2.7.1.107) (DGK) (Ca=2).
- 25 - FAD-dependent glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) from mammals (Ca=1). - Fimbrin (plastin) (Ca=2).
- Flagellar calcium-binding protein (If8) from *Trypanosoma cruzi* (Ca=1 or 2).
- Guanylate cyclase activating protein (GCAP) (Ca=3).
- Inositol phospholipid-specific phospholipase C isozymes gamma-1 and delta-1
- 30 (Ca=2) [10]. - Intestinal calcium-binding protein (ICaBPs) (Ca=2).
- MIF related proteins 8 (MRP-8 or CFAG) and 14 (MRP-14) (Ca=2).
- Myosin regulatory light chains (Ca=1). - Oncomodulin (Ca=2).
- Osteonectin (basement membrane protein BM-40) (SPARC) and proteins that contains an 'osteonectin' domain (OR1, matrix glycoprotein SC1) (see the

entry <PDOC00535>) (Ca=1). - Parvalbumins alpha and beta (Ca=2).

- Placental calcium-binding protein (18a2) (nerve growth factor induced protein 42a) (p9k) (Ca=2).

- Recoverins (visinin, hippocalcin, neurocalcin, S-modulin) (Ca=2 to 3).

5 - Reticulocalbin (Ca=4). - S-100 protein, alpha and beta chains (Ca=2).

- Sarcoplasmic calcium-binding protein (SCPs) (Ca=2 to 3).

- Sea urchin proteins Spec 1 (Ca=4), Spec 2 (Ca=4?), Lps-1 (Ca=8).

- Serine/threonine protein phosphatase rdgc (EC 3.1.3.16) from *Drosophila* (Ca=2) - Sorcin V19 from hamster (Ca=2). - Spectrin alpha chain (Ca=2).

10 - Squidulin (optic lobe calcium-binding protein) from squid (Ca=4).

- Troponins C; from skeletal muscle (Ca=4), from cardiac muscle (Ca=3), from arthropods and molluscs (Ca=2).

There has been a number of attempts [7,8] to develop patterns that pick-up EF-hand regions, but these studies were made a few years ago when not so many

15 different families of calcium-binding proteins were known. Therefore

a new pattern was developed which takes into account all published sequences. This pattern includes the complete EF-hand loop as well as the first residue which follows the loop and which seem to always be hydrophobic.

20 -Consensus pattern: D-x-[DNS]-{ILVFYW}-[DENSTG]-[DNQGHRK]-{GP}-[LIVMC]-[DENQSTAGC]-x(2)-[DE]-[LIVMFYW]

-Note: positions 1 (X), 3 (Y) and 12 (-Z) are the most conserved.

-Note: the 6th residue in an EF-hand loop is, in most cases a Gly, but the number of exceptions to this 'rule' has gradually increased and therefore the pattern should include all

25 the different residues which have been shown to exist in this position in functional Ca-binding sites.

-Note: the pattern will, in some cases, miss one of the EF-hand regions in some proteins with multiple EF-hand domains.

30 [1] Kawasaki H., Kretsinger R.H. *Protein Prof.* 2:305-490(1995).[2] Kretsinger R.H. *Cold Spring Harbor Symp. Quant. Biol.* 52:499-510(1987).

[3] Moncrief N.D., Kretsinger R.H., Goodman M. *J. Mol. Evol.* 30:522-562(1990).

[4] Nakayama S., Moncrief N.D., Kretsinger R.H. *J. Mol. Evol.* 34:416-448(1992).

[5] Heizmann C.W., Hunziker W. *Trends Biochem. Sci.* 16:98-103(1991).

[6] Kligman D., Hilt D.C. Trends Biochem. Sci. 13:437-443(1988).

[7] Strynadka N.C.J., James M.N.G.

Annu. Rev. Biochem. 58:951-98(1989).

[8] Haiech J., Sallantin J. Biochimie 67:555-560(1985).

5 [9] Chauvaux S., Beguin P., Aubert J.-P., Bhat K.M., Gow L.A., Wood T.M., Bairoch A. Biochem. J. 265:261-265(1990).

[10] Bairoch A., Cox J.A. FEBS Lett. 269:454-456(1990).

10 184. Enolase signature

Enolase (EC 4.2.1.11) is a glycolytic enzyme that catalyzes the dehydration of 2-phospho-D-glycerate to phosphoenolpyruvate [1]. It is a dimeric enzyme that requires magnesium both for catalysis and stabilizing the dimer. Enolase is probably found in all organisms that metabolize sugars. In vertebrates, there are three different tissue-specific isozymes: alpha present in most tissues, beta in muscles and gamma found only in nervous tissues. Tau-crystallin, one of the major lens proteins in some fish, reptiles and birds, has been shown [2] to be evolutionary related to enolase. As a signature pattern for enolase, the best conserved region was selected, it is located in the C-terminal third of the sequence.-

20 Consensus pattern: [LIV](3)-K-x-N-Q-I-G-[ST]-[LIV]-[ST]-[DE]-[STA]

[1] Lebioda L., Stec B., Brewer J.M. J. Biol. Chem. 264:3685-3693(1989).

[2] Wistow G., Piattigorsky J. Science 236:1554-1556(1987).

25 185. (F-actin_cap_A) F-actin capping protein alpha subunit signatures

The F-actin capping protein binds in a calcium-independent manner to the fast growing ends of actin filaments (barbed end) thereby blocking the exchange of subunits at these ends. Unlike gelsolin and severin this protein does not sever actin filaments. The F-actin capping protein is a heterodimer composed of two unrelated subunits: alpha and beta. The alpha

30 subunit is a protein of about 268 to 286 amino acid residues whose sequence is well conserved in eukaryotic species [1]. As signature patterns two highly conserved regions in the C-terminal section of the alpha subunit were selected.

Consensus pattern: V-H-[FY](2)-E-D-G-N-V

Consensus pattern: F-K-[AE]-L-R-R-x-L-P-

[1] Cooper J.A., Caldwell J.E., Gattermeir D.J., Torres M.A., Amatruda J.F., Casella J.F.
Cell Motil. Cytoskeleton 18:204-214(1991).

5

186. F-box domain

[1] Bai C, Sen P, Hofmann K, Ma L, Goebel M, Harper JW, Elledge SJ, Cell
1996;86:263-274. [2] Skowrya D, Craig KL, Tyers M, Elledge SJ, Harper JW, Cell
10 1997;91:209-219.

187. F-protein

Negative factor, (F-Protein) or Nef.

15

[1] Arold S, Franken P, Strub M-P, Hoh F, Benichou S, Benarous R, Dumas C; Medline:
98035457, The crystal structure of HIV-1 Nef protein bound to the Fyn kinase SH3 domain
suggests a role for this complex in altered T cell receptor signalling Structure 1997;5:1361-
1372.

20

Nef protein accelerates virulent progression of AIDS by its interaction with cellular proteins
involved in signal transduction and host cell activation. Nef has been shown to bind
specifically to a subset of the Src kinase family.

25 Number of members: 1013

188. (FAD_binding_2)

Fumarate reductase / succinate dehydrogenase FAD-binding site

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In bacteria two distinct, membrane-bound, enzyme complexes are responsible for the
interconversion of fumarate and succinate (EC 1.3.99.1): fumarate reductase (Frd) is used in
anaerobic growth, and succinate dehydrogenase (Sdh) is used in aerobic growth. Both
complexes consist of two main components: a membrane-extrinsic component composed of a

FAD-binding flavoprotein and an iron-sulfur protein; and an hydrophobic component composed of a membrane anchor protein and/or a cytochrome B.

- In eukaryotes mitochondrial succinate dehydrogenase (ubiquinone) (EC 1.3.5.1) is an enzyme
5 composed of two subunits: a FAD flavoprotein and and iron-sulfur protein.

The flavoprotein subunit is a protein of about 60 to 70 Kd to which FAD is covalently bound to a histidine residue which is located in the N-terminal section of the protein [1]. The sequence around that histidine is well conserved in Frd and Sdh from various bacterial and
10 eukaryotic species [2] and can be used as a signature pattern.

Consensus patternR-[ST]-H-[ST]-x(2)-A-x-G-G [H is the FAD binding site] Sequences known to belong to this class detected by the pattern ALL.

- [1] Blaut M., Whittaker K., Valdovinos A., Ackrell B.A., Gunsalus R.P., Cecchini G. J. Biol. Chem. 264:13599-13604(1989).

[2] Birch-Machin M.A., Farnsworth L., Ackrell B.A., Cochran B., Jackson S., Bindoff L.A., Aitken A., Diamond A.G., Turnbull D.M. J. Biol. Chem. 267:11553-11558(1992).

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189. Fatty acid desaturases signatures (FA_desaturase)

Fatty acid desaturases (EC 1.14.99.-) are enzymes that catalyze the insertion of a double bond at the delta position of fatty acids. There seems to be two distinct families of fatty acid desaturases which do not seem to be evolutionary related. Family 1 is composed of: -

- 25 Stearoyl-CoA desaturase (SCD) (EC 1.14.99.5) [1]. SCD is a key regulatory enzyme of unsaturated fatty acid biosynthesis. SCD introduces a cis double bond at the delta(9) position of fatty acyl-CoA's such as palmitoleoyl- and oleoyl-CoA. SCD is a membrane-bound enzyme that is thought to function as a part of a multienzyme complex in the endoplasmic reticulum of vertebrates and fungi. As a signature pattern for this family a conserved region
30 in the C-terminal part of these enzymes was selected, this region is rich in histidine residues and in aromatic residues. Family 2 is composed of: - Plants stearoyl-acyl-carrier-protein desaturase (EC 1.14.99.6) [2], these enzymes catalyze the introduction of a double bond at the delta(9) position of stearoyl-ACP to produce oleoyl-ACP. This enzyme is responsible for the conversion of saturated fatty acids to unsaturated fatty acids in the synthesis of vegetable

oils. - Cyanobacteria desA [3] an enzyme that can introduce a second cis double bond at the delta(12) position of fatty acid bound to membranes glycerolipids. DesA is involved in chilling tolerance; the phase transition temperature of lipids of cellular membranes being dependent on the degree of unsaturation of fatty acids of the membrane lipids. As a signature pattern for this family a conserved region in the C-terminal part of these enzymes was selected.

Consensus pattern: G-E-x-[FY]-H-N-[FY]-H-H-x-F-P-x-D-Y-

Consensus pattern: [ST]-[SA]-x(3)-[QR]-[LI]-x(5,6)-D-Y-x(2)-[LIVMFYW]-[LIVM]-[DE]-

[1] Kaestner K.H., Ntambi J.M., Kelly T.J. Jr., Lane M.D. J. Biol. Chem. 264:14755-14761(1989).

[2] Shanklin J., Somerville C.R. Proc. Natl. Acad. Sci. U.S.A. 88:2510-2514(1991).

[3] Wada H., Gombos Z., Murata N. Nature 347:200-203(1990).

190. Fructose-1-6-bisphosphatase active site (FBPase)

Fructose-1,6-bisphosphatase (EC 3.1.3.11) (FBPase) [1], a regulatory enzyme in gluconeogenesis, catalyzes the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate. It is involved in many different metabolic pathways and found in most organisms. Sedoheptulose-1,7-bisphosphatase (EC 3.1.3.37) (SBPase) [2] is an enzyme found plant chloroplast and in photosynthetic bacteria that catalyzes the hydrolysis of sedoheptulose 1,7-bisphosphate to sedoheptulose 7-phosphate, a step in the Calvin's reductive pentose phosphate cycle. It is functionally and structurally related to FBPase. In mammalian FBPase, a lysine residue has been shown to be involved in the catalytic mechanism [3]. The region around this residue is highly conserved and can be used as a signature pattern for FBPase and SBPase. It must be noted that, in some bacterial FBPase sequences, the active site lysine is replaced by an arginine

Consensus pattern: [AG]-[RK]-L-x(1,2)-[LIV]-[FY]-E-x(2)-P-[LIVM]-[GSA] [K/R is the active site residue]-

[1] Benkovic S.J., DeMaine M.M. Adv. Enzymol. 53:45-82(1982).

- [2] Raines C.A., Lloyd J.C., Willingham N.M., Potts S., Dyer T.A. Eur. J. Biochem. 205:1053-1059(1992).
- [3] Ke H., Thorpe C.M., Seaton B.A., Lipscomb W.N., Marcus F. J. Mol. Biol. 212:513-539(1989).

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191. FGGY family of carbohydrate kinases signatures *

It has been shown [1] that four different type of carbohydrate kinases seem to be evolutionary related. These enzymes are: - L-fucolokinase (EC 2.7.1.51) (gene fucK). - Gluconokinase (EC 2.7.1.12) (gene gntK). - Glycerokinase (EC 2.7.1.30) (gene glpK). - Xylulokinase (EC 2.7.1.17) (gene xylB). - L-xylulose kinase (EC 2.7.1.53) (gene lyxK). These enzymes are proteins of from 480 to 520 amino acid residues. As consensus patterns for this family of kinases two conserved regions were selected, one in the central section, the other in the C-terminal section.

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Consensus pattern: [MFYGS]-x-[PST]-x(2)-K-[LIVMFYW]-x-W-[LIVMF]-x-[DENQTKR]-[ENQH]-

Consensus pattern: [GSA]-x-[LIVMFYW]-x-G-[LIVM]-x(7,8)-[HDENQ]-[LIVMF]-x(2)-[AS]-[STAIVM]-[LIVMFY]-[DEQ]-

15

[1] Reizer A., Deutscher J., Saier M.H. Jr., Reizer J. Mol. Microbiol. 5:1081-1089(1991).

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192. FKBP-type peptidyl-prolyl cis-trans isomerase signatures/profile (FKBP)

FKBP [1,2,3] is the major high-affinity binding protein, in vertebrates, for the immunosuppressive drug FK506. It exhibits peptidyl-prolyl cis-trans isomerase activity (EC 5.2.1.8) (PPIase or rotamase). PPIase is an enzyme that accelerates protein folding by catalyzing the cis-trans isomerization of proline imidic peptide bonds in oligopeptides [4]. At least three different forms of FKBP are known in mammalian species: - FKBP-12, which is cytosolic and inhibited by both FK506 and rapamycin. - FKBP-13, which is membrane associated and inhibited by both FK506 and rapamycin. - FKBP-25, which is preferentially inhibited by rapamycin. These forms of FKBP are evolutionary related and show extensive similarities [5,6,7] with the following proteins: - Fungal FKBP. - Mammalian hsp binding immunophilin (HBI) (also called p59). HBI is a protein which binds to hsp90 and contains

25

30

two FKBP-like domains in its N- terminal section - the first of which seems to be functional.

- The C-terminal part of the cell-surface protein mip from *Legionella*; a protein associated with macrophage infection by an unknown mechanism. - *Escherichia coli* slyD [8], a protein with a N-terminal FKBP domain followed by an histidine-rich metal-binding domain. -

- 5 *Escherichia coli* fkpA. - *Escherichia coli* fklB (FKBP22). - *Escherichia coli* slpA. - Bacterial trigger factor (Tig). - *Streptomyces hygroscopicus* and *chrysomallus* FK506-binding protein. - *Chlamydia trachomatis* 27 Kd membrane protein. - *Neisseria meningitidis* strain C114 PPIase. - Probable PPIases from *Haemophilus influenzae* (HI0754), *Methanococcus jannaschii* (MJ0278 and MJ0825), *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*.
- 10 Two signature patterns for these proteins were developed. One is based on a conserved region in the N-terminus of FKBP, the other is located in the central section. The profile for FKBP spans the complete domain.

Consensus pattern: [LIVMC]-x-[YF]-x-[GVL]-x(1,2)-[LFT]-x(2)-G-x(3)-[DE]-[STAEQK]-[STAN]-

- 15 Consensus pattern: [LIVMFY]-x(2)-[GA]-x(3,4)-[LIVMF]-x(2)-[LIVMFHK]-x(2)-G-x(4)-[LIVMF]-x(3)-[PSGAQ]-x(2)-[AG]-[FY]-G--

- [1] Tropschug M., Wachter E., Mayer S., Schoenbrunner E.R., Schmid F.X. *Nature* 346:674-677(1990).
- 20 [2] Stein R.L. *Curr. Biol.* 1:234-236(1991).
- [3] Siekierka J.J., Widerrecht G., Greulich H., Boulton D., Hung S.H.Y., Cryan J., Hodges P.J., Sigal N.H. *J. Biol. Chem.* 265:21011-21015(1990).
- [4] Fischer G., Schmid F.X. *Biochemistry* 29:2205-2212(1990).
- 25 [5] Trandinh C.C., Pao G.M., Saier M.H. Jr. *FASEB J.* 6:3410-3420(1992).
- [6] Galat A. *Eur. J. Biochem.* 216:689-707(1993).
- [7] Hacker J., Fischer G. *Mol. Microbiol.* 10:445-456(1993).
- [8] Wuefling C., Lomardero J., Plueckthun A. *J. Biol. Chem.* 269:2895-2901(1994).

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193. MAPEG family (aka: FLAP/GST2/LTC4S family signature)

The following mammalian proteins are evolutionary related [1]:

- Leukotriene C4 synthase (EC 2.5.1.37) (gene LTC4S), an enzyme that catalyzes the production of LTC4 from LTA4.

- Microsomal glutathione S-transferase II (EC 2.5.1.18) (GST-II) (gene GST2), an enzyme that can also produces LTC4 from LTA4.
- 5-lipoxygenase activating protein (gene FLAP), a protein that seems to be required for the activation of 5-lipoxygenase.

These are proteins of 150 to 160 residues that contain three transmembrane segments. As a signature pattern, a conserved region between the first and second transmembrane domains was selected.

Consensus pattern: G-x(3)-F-E-R-V-[FY]-x-A-[NQ]-x-N-C

[1] Jakobsson P.-J., Mancini J.A., Ford-Hutchinson A.W. J. Biol. Chem. 271:22203-22210(1996).

194. FMN-dependent alpha-hydroxy acid dehydrogenases active site (FMN_dh)

A number of oxidoreductases that act on alpha-hydroxy acids and which are FMN-containing flavoproteins have been shown [1,2,3] to be structurally related; these enzymes are: - Lactate dehydrogenase (EC 1.1.2.3), which consists of a dehydrogenase domain and a heme-binding domain called cytochrome b2 and which catalyzes the conversion of lactate into pyruvate. - Glycolate oxidase (EC 1.1.3.15) ((S)-2-hydroxy-acid oxidase), a peroxisomal enzyme that catalyzes the conversion of glycolate and oxygen to glyoxylate and hydrogen peroxide. - Long chain alpha-hydroxy acid oxidase from rat (EC 1.1.3.15), a peroxisomal enzyme. - Lactate 2-monooxygenase (EC 1.13.12.4) (lactate oxidase) from Mycobacterium smegmatis, which catalyzes the conversion of lactate and oxygen to acetate, carbon dioxide and water. - (S)-mandelate dehydrogenase from Pseudomonas putida (gene mdlB), which catalyzes the reduction of (S)-mandelate to benzoylformate. The first step in the reaction mechanism of these enzymes is the abstraction of the proton from the alpha-carbon of the substrate producing a carbanion which can subsequently attach to the N5 atom of FMN. A conserved histidine has been shown [4] to be involved in the removal of the proton. The region around this active site residue is highly conserved and contains an arginine residue which is involved in substrate binding.

Consensus pattern: S-N-H-G-[AG]-R-Q [H is the active site residue] [R is a substrate-binding residue]-

[1] Giegel D.A., Williams C.H. Jr., Massey V. J. Biol. Chem. 265:6626-6632(1990).

[2] Tsou A.Y., Ransom S.C., Gerlt J.A., Buechter D.D., Babbitt P.C., Kenyon G.L.
Biochemistry 29:9856-9862(1990).

[3] Le K.H.D., Lederer F. J. Biol. Chem. 266:20877-20880(1991).

5 [4] Lindqvist Y., Branden C.-I. J. Biol. Chem. 264:3624-3628(1989).

195. Flavin-binding monooxygenase-like (FMO-like)

This family includes FMO proteins, cyclohexanone monooxygenase

10

196. (FPGS)

Folypolyglutamate synthase signatures (aka Mur_ligase)

15 Folypolyglutamate synthase (EC 6.3.2.17) (FPGS) [1] is the enzyme of folate metabolism
that catalyzes ATP-dependent addition of glutamate moieties to tetrahydrofolate.

Its sequence is moderately conserved between prokaryotes (gene folC) and eukaryotes. We
developed two signature patterns based on the conserved regions which are rich in glycine
20 residues and could play a role in the catalytical activity and/or in substrate binding.

Consensus pattern [LIVMFY]-x-[LIVM]-[STAG]-G-T-[NK]-G-K-x-[ST]-x(7)-[LIVM](2)-
x(3)-[GSK] Sequences known to belong to this class detected by the pattern ALL.

25 Consensus pattern[LIVMFY](2)-E-x-G-[LIVM]-[GA]-G-x(2)-D-x-[GST]-x-[LIVM](2)
Sequences known to belong to this class detected by the pattern ALL.

[1] Shane B., Garrow T., Brenner A., Chen L., Choi Y.J., Hsu J.C., Stover P. Adv. Exp.
Med. Biol. 338:629-634(1993).

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197. FYVE zinc finger

The FYVE zinc finger is named after four proteins that it has been found in: Fab1, YOTB/ZK632.12, Vac1, and EEA1. The FYVE finger has been shown to bind two Zn++ ions [1]. The FYVE finger has eight potential zinc coordinating cysteine positions. Many members of this family also include two histidines in a motif R+HHC+XCG, where + represents a charged residue and X any residue. Members were included which do not conserve these histidine residues but are clearly related.

[1] Stenmark H, Aasland R, Toh BH, D'Arrigo A, J Biol Chem 1996;271:24048-24054. [2] Gaullier JM, Simonsen A, D'Arrigo A, Bremnes B, Stenmark H, Aasland R, Nature 1998;394:432-433.

198. F_actin_cap_B

F-actin capping protein beta subunit signature

The F-actin capping protein binds in a calcium-independent manner to the fast growing ends of actin filaments (barbed end) thereby blocking the exchange of subunits at these ends. Unlike gelsolin and severin this protein does not sever actin filaments. The F-actin capping protein is a heterodimer composed of two unrelated subunits: alpha and beta.

The beta subunit is a protein of about 280 amino acid residues whose sequence is well conserved in eukaryotic species [1]. As a signature pattern a conserved hexapeptide in the N-terminal section of the beta subunit was selected.

Consensus pattern: C-D-Y-N-R-D Sequences known to belong to this class detected by the pattern ALL.

[1] Amatruda J.F., Cannon J.F., Tatchell K., Hug C., Cooper J.A. Nature 344:352-354(1990).

199. Isopenicillin N synthetase signatures (Fe_Asc_oxidored)

Isopenicillin N synthetase (IPNS) [1,2] is a key enzyme in the biosynthesis of penicillin and cephalosporin. In the presence of oxygen, it removes iron and ascorbate, four hydrogen atoms from L-(alpha-aminoadipyl)-L-cysteinyld-valine to form the azetidinone and thiazolidine rings of isopenicillin. IPNS is an enzyme of about 330 amino-acid residues. Two cysteines

are conserved in fungal and bacterial IPNS sequences; these may be involved in iron-binding and/or substrate-binding. Cephalosporium acremonium DAOCS/DACS [3] is a bifunctional enzyme involved in cephalosporin biosynthesis. The DAOCS domain, which is structurally related to IPNS, catalyzes the step from penicillin N to deacetoxy-cephalosporin C - used as a substrate by DACS to form deacetylcephalosporin C. Streptomyces clavuligerus possesses a monofunctional DAOCS enzyme (gene cefE) [4] also related to IPNS. Two signature patterns for these enzymes were derived, centered around the conserved cysteine residues.

Consensus pattern: [RK]-x-[STA]-x(2)-S-x-C-Y-[SL]-

Consensus pattern: [LIVM](2)-x-C-G-[STA]-x(2)-[STAG]-x(2)-T-x-[DNG]-

[1] Martin J.F. Trends Biotechnol. 5:306-308(1987).

[2] Chen G., Shiffman D., Mevarech M., Aharonowitz Y. Trends Biotechnol. 8:105-111(1990).

[3] Samson S.M., Dotzlaw J.E., Slisz M.L., Becker G.W., van Frank R.M., Veal L.E., Yeh W.K., Miller J.R., Queener S.W., Ingolia T.D. Bio/Technology 5:1207-1214(1987).

[4] Kovacevic S., Weigel B.J., Tobin M.B., Ingolia T.D., Miller J.R. J. Bacteriol. 171:754-760(1989).

200. Fibrillarlin signature

Fibrillarlin [1] is a component of a nucleolar small nuclear ribonucleoprotein(SnRNP) particle thought to participate in the first step of the processing of pre-rRNA. In mammals, fibrillarlin is associated with the U3, U8 and U13small nuclear RNAs [2]. Fibrillarlin is an extremely well conserved protein of about 320 amino acid residues. Structurally it consists of three different domains: - An N-terminal domain of about 80 amino acids which is very rich in glycine and contains a number of dimethylated arginine residues (DMA). - A central domain of about 90 residues which resembles that of RNA-binding proteins and contains an octameric sequence similar to the RNP-2 consensus found in such proteins. - A C-terminal alpha-helical domain. A protein evolutionary related to fibrillarlin has been found [3] in archaebacteria such as Methanococcus vannielii or voltae. This protein (gene flpA) is involved in pre-rRNA processing. It lacks the Gly/Arg-rich N-terminal domain. As a signature pattern, a region was selected that starts with and encompasses the RNP-2 like octapeptide sequence.

Consensus pattern: [GST]-[LIVMAP]-V-Y-A-[IV]-E-[FY]-[SA]-x-R-x(2)-R-[DE] -

[1] Aris J.P., Blobel G. Proc. Natl. Acad. Sci. U.S.A. 88:931-935(1991).

5 [2] Bandziulis R.J., Swanson M.S., Dreyfuss G. Genes Dev. 3:431-437(1989).

[3] Agha-Amiri K. J. Bacteriol. 176:2124-2127(1994).

201. Filamin/ABP280 repeat

10 [1] Fucini P, Renner C, Herberhold C, Noegel AA, Holak TA, Nat Struct Biol
1997;4:223-230.

202. Fucosyl transferase

15 This family of Fucosyltransferases are the enzymes transferring
fucose from GDP-Fucose to GlcNAc in an alpha1,3 linkage [1].

[1] Breton C, Oriol R, Imberty A; Glycobiology 1998;8:87-94.

20 203. 2Fe-2S ferredoxins, iron-sulfur binding region signature (fer2A)

Ferredoxins [1] are a group of iron-sulfur proteins which mediate electron transfer in a wide variety of metabolic reactions. Ferredoxins can be divided into several subgroups depending upon the physiological nature of the iron sulfur cluster(s) and according to sequence similarities. One of these subgroups are the 2Fe-2S ferredoxins, which are proteins or
25 domains of around one hundred amino acid residues that bind a single 2Fe-2S iron-sulfur cluster. The proteins that are known [2] to belong to this family are listed below. - Ferredoxin from photosynthetic organisms; namely plants and algae where it is located in the chloroplast or cyanelle; and cyanobacteria. - Ferredoxin from archaeobacteria of the Halobacterium genus. - Ferredoxin IV (gene pftA) and V (gene fdxD) from Rhodobacter capsulatus. - Ferredoxin in
30 the toluene degradation operon (gene xylT) and naphthalene degradation operon (gene nahT) of Pseudomonas putida. - Hypothetical Escherichia coli protein yfaE. - The N-terminal domain of the bifunctional ferredoxin/ferredoxin reductase electron transfer component of the benzoate 1,2-dioxygenase complex (gene benC) from Acinetobacter calcoaceticus, the toluene 4-monooxygenase complex (gene tmoF), the toluate 1,2-dioxygenase system (gene

xylZ), and the xylene monooxygenase system (gene xylA) from *Pseudomonas*. - The N-terminal domain of phenol hydroxylase protein p5 (gene dmpP) from *Pseudomonas Putida*. - The N-terminal domain of methane monooxygenase component C (gene mmoC) from *Methylococcus capsulatus*. - The C-terminal domain of the vanillate degradation pathway protein vanB in a *Pseudomonas* species. - The N-terminal domain of bacterial fumarate reductase iron-sulfur protein (gene frdB). - The N-terminal domain of CDP-6-deoxy-3,4-glucose reductase (gene ascD) from *Yersinia pseudotuberculosis*. - The central domain of eukaryotic succinate dehydrogenase (ubiquinone) iron-sulfur protein. - The N-terminal domain of eukaryotic xanthine dehydrogenase. - The N-terminal domain of eukaryotic aldehyde oxidase. In the 2Fe-2S ferredoxins, four cysteine residues bind the iron-sulfur cluster. Three of these cysteines are clustered together in the same region of the protein. Our signature pattern spans that iron-sulfur binding region.

Consensus pattern: C-{C}-{C}-[GA]-{C}-C-[GAST]-{CPDEKRHFYW}-C [The three C's are 2Fe-2S ligands]-

[1] Meyer J. Trends Ecol. Evol. 3:222-226(1988).[2] Harayama S., Polissi A., Rekik M. FEBS Lett. 285:85-88(1991).

Adrenodoxin family, iron-sulfur binding region signature (fer2B)

Ferredoxins [1] are a group of iron-sulfur proteins which mediate electron transfer in a wide variety of metabolic reactions. Ferredoxins can be divided into several subgroups depending upon the physiological nature of the iron sulfur cluster(s) and according to sequence similarities. One family of ferredoxins groups together the following proteins that all bind a single 2Fe-2S iron-sulfur cluster: - Adrenodoxin (ADX) (adrenal ferredoxin), a vertebrate mitochondrial protein which transfers electrons from adrenodoxin reductase to cytochrome P450_{sec}, which is involved in cholesterol side chain cleavage. - Putidaredoxin (PTX), a *Pseudomonas putida* protein which transfers electrons from putidaredoxin reductase to cytochrome P450_{cam}, which is involved in the oxidation of camphor. - Terpredoxin [2], a *Pseudomonas* protein which transfers electrons from terpredoxin reductase to cytochrome P450_{terp}, which is involved in the oxidation of alpha-terpineol. - Rhodocoxin [3], a *Rhodococcus* protein which transfers electrons from rhodocoxin reductase to cytochrome CYP116 (thcB), which is involved in the degradation of thiocarbamate herbicides. - *Escherichia coli* ferredoxin (gene fdx) [4] whose exact function is not yet known. -

Rhodobacter capsulatus ferredoxin VI [5], which may transfer electrons to a yet uncharacterized oxygenase. - Caulobacter crescentus ferredoxin (gene fdxB) [6]. In these proteins, four cysteine residues bind the iron-sulfur cluster. Three of these cysteines are clustered together in the same region of the protein. Our signature pattern spans that iron-sulfur binding region.

Consensus pattern: C-x(2)-[STAQ]-x-[STAMV]-C-[STA]-T-C-[HR] [The three C's are 2Fe-2S ligands]-

[1] Meyer J. Trends Ecol. Evol. 3:222-226(1988).

[2] Peterson J.A., Lu J.-Y., Geisselsoder J., Graham-Lorence S., Carmona C., Witney F., Lorence M.C. J. Biol. Chem. 267:14193-14203(1992).

[3] Nagy I., Schoofs G., Compennolle F., Proost P., Vanderleyden J., De Mot R. J. Bacteriol. 177:676-687(1995).

[4] Ta D.T., Vickery L.E. J. Biol. Chem. 267:11120-11125(1992).

[5] Naud I., Vincon M., Garin J., Gaillard J., Forest E., Jouanneau Y. Eur. J. Biochem. 222:933-939(1994).

[6] Amemiya K EMBL/Genbank: X51607.

204. 4Fe-4S ferredoxins, iron-sulfur binding region signature (fer4)

Ferredoxins [1] are a group of iron-sulfur proteins which mediate electron transfer in a wide variety of metabolic reactions. Ferredoxins can be divided into several subgroups depending upon the physiological nature of the iron-sulfur cluster(s). One of these subgroups are the 4Fe-4S ferredoxins, which are found in bacteria and which are thus often referred as 'bacterial-type' ferredoxins. The structure of these proteins [2] consists of the duplication of a domain of twenty six amino acid residues; each of these domains contains four cysteine residues that bind to a 4Fe-4S center. A number of proteins have been found [3] that include one or more 4Fe-4S binding domains similar to those of bacterial-type ferredoxins. These proteins are listed below (references are only provided for recently determined sequences). - The iron-sulfur proteins of the succinate dehydrogenase and the fumarate reductase complexes (EC 1.3.99.1). These enzyme complexes, which are components of the tricarboxylic acid cycle, each contain three subunits: a flavoprotein, an iron-sulfur protein, and a b-type cytochrome. The iron-sulfur proteins contain three different iron-sulfur centers:

a 2Fe-2S, a 3Fe-3S and a 4Fe-4S. - *Escherichia coli* anaerobic glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) This enzyme is composed of three subunits: A, B, and C. The C subunit seems to be an iron-sulfur protein with two ferredoxin-like domains in the N-terminal part of the protein. - *Escherichia coli* anaerobic dimethyl sulfoxide reductase. The B subunit of this enzyme (gene *dmsB*) is an iron-sulfur protein with four 4Fe-4S ferredoxin-like domains. - *Escherichia coli* formate hydrogenlyase. Two of the subunits of this oligomeric complex (genes *hycB* and *hycF*) seem to be iron-sulfur proteins that each contain two 4Fe-4S ferredoxin-like domains. - *Methanobacterium formicicum* formate dehydrogenase (EC 1.2.1.2). This enzyme is used by the archaeobacteria to grow on formate. The beta chain of this dimeric enzyme probably binds two 4Fe-4S centers. - *Escherichia coli* formate dehydrogenases N and O (EC 1.2.1.2). The beta chain of these two enzymes (genes *fdnH* and *fdoH*) are iron-sulfur proteins with four 4Fe-4S ferredoxin-like domains. - *Desulfovibrio* periplasmic [Fe] hydrogenase (EC 1.18.99.1). The large chain of this dimeric enzyme binds three 4Fe-4S centers, two of which are located in the ferredoxin-like N-terminal region of the protein. - *Methanobacterium thermoautrophicum* methyl viologen-reducing hydrogenase subunit *mvhB*, which contains six tandemly repeated ferredoxin-like domains and which probably binds twelve 4Fe-4S centers. - *Salmonella typhimurium* anaerobic sulfite reductase (EC 1.8.1.-) [4]. Two of the subunits of this enzyme (genes *asrA* and *asrC*) seem to both bind two 4Fe-4S centers. - A Ferredoxin-like protein (gene *fixX*) from the nitrogen-fixation genes locus of various *Rhizobium* species, and one from the *Nif*-region of *Azotobacter* species. - The 9 Kd polypeptide of chloroplast photosystem I [5] (gene *psaC*). This protein contains two low potential 4Fe-4S centers, referred as the A and B centers. - The chloroplast *frxB* protein which is predicted to carry two 4Fe-4S centers. - An ferredoxin from a primitive eukaryote, the enteric amoeba *Entamoeba histolytica*. - *Escherichia coli* hypothetical protein *yjjW*, a protein with a N-terminal region belonging to the radical activating enzymes family (see <PDOC00834>) and two potential 4Fe-4S centers. The pattern of cysteine residues in the iron-sulfur region is sufficient to detect this class of 4Fe-4S binding proteins.

Consensus pattern: C-x(2)-C-x(2)-C-x(3)-C-[PEG] [The four C's are 4Fe-4S ligands]-

- [1] Meyer J. Trends Ecol. Evol. 3:222-226(1988).
- [2] Otake E., Ooi T. J. Mol. Evol. 26:257-267(1987).
- [3] Beinert H. FASEB J. 4:2483-2492(1990).
- [4] Huang C.J., Barrett E.L. J. Bacteriol. 173:1544-1553(1991).

[5] Knaff D.B. Trends Biochem. Sci. 13:460-461(1988).

205. NifH/frxC family signatures (fer4_NifH)

- 5 Nitrogenase (EC 1.18.6.1) [1] is the enzyme system responsible for biological nitrogen fixation. Nitrogenase is an oligomeric complex which consists of two components: component 1 which contains the active site for the reduction of nitrogen to ammonia and component 2 (also called the iron protein). Component 2 is a homodimer of a protein (gene nifH) which binds a single 4Fe-4S iron sulfur cluster [2]. In the nitrogen fixation process nifH
- 10 is first reduced by a protein such as ferredoxin; the reduced protein then transfers electrons to component 1 with the concomitant consumption of ATP. A number of proteins are known to be evolutionary related to nifH. These proteins are: - Chloroplast encoded frxC (or chlL) protein [3]. FrxC is encoded on the chloroplast genome of some plant species, its exact function is not known, but it could act as an electron carrier in the conversion of
- 15 protochlorophyllide to chlorophyllide. - Rhodobacter capsulatus proteins bchL and bchX [4]. These proteins are also likely to play a role in chlorophyll synthesis. There are a number of conserved regions in the sequence of these proteins: in the N-terminal section there is an ATP-binding site motif 'A' (P-loop) and in the central section there are two conserved cysteines which have been shown, in nifH, to be the ligands of the 4Fe-4S cluster. Two
- 20 signatures patterns that correspond to the regions around these cysteines were developed.

Consensus pattern: E-x-G-G-P-x(2)-[GA]-x-G-C-[AG]-G [C binds the iron-sulfur center]-

Consensus pattern: D-x-L-G-D-V-V-C-G-G-F-[AG]-x-P [C binds the iron-sulfur center]-

- 25 [1] Pau R.N. Trends Biochem. Sci. 14:183-186(1989).
 [2] Georgiadis M.M., Komiya H., Chakrabarti P., Woo D., Kornuc J.J., Rees D.C. Science 257:1653-1659(1992).
 [3] Fujita Y., Takahashi Y., Kohchi T., Ozeki H., Ohyama K., Matsubara H. Plant Mol. Biol. 13:551-561(1989).
 30 [4] Burke D.H., Alberti M., Hearst J.E. J. Bacteriol. 175:2407-2413(1993).

206. Ferritin iron-binding regions signatures

Ferritin [1,2] is one of the major non-heme iron storage proteins. It consists of a mineral core of hydrated ferric oxide, and a multi-subunit protein shell which englobes the former and assures its solubility in an aqueous environment. In animals the protein is mainly cytoplasmic and there are generally two or more genes that encode for closely related subunits (in mammals there are two subunits which are known as H(eavy) and L(ight)). In plants ferritin is found in the chloroplast [3]. There are a number of well conserved regions in the sequence of ferritins. Two of these regions to develop signature patterns were selected. The first pattern is located in the central part of the sequence of ferritin and it contains three conserved glutamate which are thought to be involved in the binding of iron. The second pattern is located in the C-terminal section, it corresponds to a region which forms a hydrophilic channel through which small molecules and ions can gain access to the central cavity of the molecule; this pattern also includes conserved acidic residues which are potential metal-binding sites.

Consensus pattern: E-x-[KR]-E-x(2)-E-[KR]-[LF]-[LIVMA]-x(2)-Q-N-x-R-x-G-R [The 3 E's are potential iron ligands]-

Consensus pattern: D-x(2)-[LIVMF]-[STAC]-[DH]-F-[LI]-[EN]-x(2)-[FY]-L-x(6)-[LIVM]-[KN] [The second D and the E are potential iron ligands]-

[1] Crichton R.R., Charleatoux-Wauters M. Eur. J. Biochem. 164:485-506(1987).

[2] Theil E.C. Annu. Rev. Biochem. 56:289-315(1987).

[3] Ragland M., Briat J.-F., Gagnon J., Lahlhere J.-P., Massenet O., Theil E.C. J. Biol. Chem. 265:18339-18344(1990).

207. Intermediate filaments signature (filament)

Intermediate filaments (IF) [1,2,3] are proteins which are primordial components of the cytoskeleton and the nuclear envelope. They generally form filamentous structures 8 to 14 nm wide. IF proteins are members of a very large multigene family of proteins which has been subdivided in five major subgroups: - Type I: Acidic cytokeratins. - Type II: Basic cytokeratins. - Type III: Vimentin, desmin, glial fibrillary acidic protein (GFAP), peripherin, and plasticin. - Type IV: Neurofilaments L, H and M, alpha-internexin and nestin. - Type V: Nuclear lamins A, B1, B2 and C. All IF proteins are structurally similar in that they consist of: a central rod domain comprising some 300 to 350 residues which is arranged in coiled-coiled alpha-helices, with at least two short characteristic interruptions; a N-terminal non-

helical domain (head) of variable length; and a C-terminal domain (tail) which is also non-helical, and which shows extreme length variation between different IF proteins. While IF proteins are evolutionary and structurally related, they have limited sequence homologies except in several regions of the rod domain. A conserved region at the C-terminal extremity of the rod domain was used as a sequence pattern for this class of proteins.

Consensus pattern: [IV]-x-[TACI]-Y-[RKH]-x-[LM]-L-[DE]-

[1] Quinlan R., Hutchison C., Lane B. Protein Prof. 2:801-952(1995).

[2] Steiner P.M., Roop D.R. Annu. Rev. Biochem. 57:593-625(1988).

[3] Stewart M. Curr. Opin. Cell Biol. 2:91-100(1990).

208. Flavodoxin signature

Flavodoxins [1,El] are electron-transfer proteins that function in various electron transport systems. Flavodoxins bind one FMN molecule, which serves as a redox-active prosthetic group. Flavodoxins are functionally interchangeable with ferredoxins. They have been isolated from prokaryotes, cyanobacteria, and some eukaryotic algae. The signature pattern for these proteins is derived from a conserved region in their N-terminal section, this region is involved in the binding of the FMN phosphate group.

Consensus pattern: [LIV]-[LIVFY]-[FY]-x-[ST]-x(2)-[AGC]-x-T-x(3)-A-x(2)-[LIV]-

[1] Wakabayashi S., Kimura K., Matsubara H., Rogers L.J. Biochem. J. 263:981-984(1989).

209. Growth factor and cytokines receptors family signatures (fn3)

A number of receptors for lymphokines, hematopoietic growth factors and growth hormone-related molecules have been found [1 to 5] to share a common binding domain. Receptors known to belong to this family are: - Cytokine receptor common beta chain. This chain is common to the IL-3, IL-5 and GM-CSF receptors. - Cytokine receptor common gamma chain. This chain is common to the IL-2, IL-4, IL-7 and IL-13 receptors. - Ciliary neurotrophic factor receptor (CNTFR). - Erythropoietin receptor (EPOR). - Granulocyte colony-stimulating factor receptor (G-CSFR). - Granulocyte-macrophage colony-stimulating

factor receptor alpha chain (GM-CSFR). - Interleukin-2 receptor beta chain (IL2R-beta). - Interleukin-3 receptor alpha chain (IL3R). - Interleukin-4 receptor alpha chain (IL4R). - Interleukin-5 receptor alpha chain (IL5R). - Interleukin-6 receptor (IL6R). - Interleukin-7 receptor alpha chain (IL7R). - Interleukin-9 receptor (IL9R). - Growth hormone receptor (GRHR). - Prolactin receptor (PRLR). - Thrombopoietin receptor (TPOR). The conserved region constitutes all or part of the extracellular ligand-binding region and is about 200 amino acid residues long. In the N-terminal of this domain there are two pairs of cysteines known, in the growth hormone receptor, to be involved in disulfide bonds. +-----+
 -----xxxxxxx-----+ | C C C C Extracellular XXXXXXXX Cytoplasmic | +-
 10 | |-----| |-----+ |||| Transmembrane ++ +-
 + Two patterns to detect this family of receptors were used. The first one is derived from the first N-terminal disulfide loop, the second is a tryptophan-rich pattern located at the C-terminal extremity of the extracellular region.

15 Consensus pattern: C-[LVFYR]-x(7,8)-[STIVDN]-C-x-W [The two C's are linked by a disulfide bond]-

Consensus pattern: [STGL]-x-W-[SG]-x-W-S-

[1] Bazan J.F. Biochem. Biophys. Res. Commun. 164:788-795(1989).

20 [2] Bazan J.F. Proc. Natl. Acad. Sci. U.S.A. 87:6934-6938(1990).

[3] Cosman D., Lyman S.D., Idzerda R.L., Beckmann M.P., Park L.S., Goodwin R.G., March C.J. Trends Biochem. Sci. 15:265-270(1990).

[4] d'Andrea A.D., Fasman G.D., Lodish H.F. Cell 58:1023-1024(1989).

[5] d'Andrea A.D., Fasman G.D., Lodish H.F. Curr. Opin. Cell Biol. 2:648-651(1990).

25 210. Phosphoribosylglycinamide formyltransferase active site (formyl_transf)

Phosphoribosylglycinamide formyltransferase (EC 2.1.2.2) (GART) [1] catalyzes the third step in de novo purine biosynthesis, the transfer of a formyl group to 5'.

30 phosphoribosylglycinamide. In higher eukaryotes, GART is part of a multifunctional enzyme polypeptide that catalyzes three of the steps of purine biosynthesis. In bacteria, plants and yeast, GART is a monofunctional protein of about 200 amino-acid residues. In the Escherichia coli enzyme, an aspartic acid residue has been shown to be involved in the catalytic mechanism. The region around this active site residue is well conserved in GART

from prokaryotic and eukaryotic sources and can be used as a signature pattern. Mammalian formyltetrahydrofolate dehydrogenase (EC 1.5.1.6) [2] is a cytosolic enzyme responsible for the NADP-dependent decarboxylative reduction of 10-formyltetrahydrofolate into tetrahydrofolate. It is a protein of about 900 amino acids consisting of three domains; the N-terminal domain (200 residues) is structurally related to GARTs. *Escherichia coli* methionyl-tRNA formyltransferase (EC 2.1.2.9) (gene *fmt*) [3] is the enzyme responsible for modifying the free amino group of the aminoacyl moiety of methionyl- A (fMet). The central part of *fmt* seems to be evolutionary related to GART's active site region.

- 10 Consensus pattern: G-x-[STM]-[IVT]-x-[FYWVO]-[VMAT]-x-[DEVN]-x-[LIVMY]-D-x-G-x(2)-[LIVT]-x(6)-[LIVM] [D is the active site residue] -

[1] Inglese J., Smith J.M., Benkovic S.J. *Biochemistry* 29:6678-6687(1990).

[2] Cook R.J., Lloyd R.S., Wagner C. J. *Biol. Chem.* 266:4965-4973(1991).

- 15 [3] Guillon J.-M., Mechulam Y., Schmitter J.-M., Blanquet S., Fayat G. J. *Bacteriol.* 174:4294-4301(1992).

211. G10 protein signatures

- 20 A *Xenopus* protein known as G10 [1] has been found to be highly conserved in a wide range of eukaryotic species. The function of G10 is still unknown. G10 is a protein of about 17 to 18 Kd (143 to 157 residues) which is hydrophilic and whose C-terminal half is rich in cysteines and could be involved in metal-binding. As signature patterns, two of these cysteine-rich segments were selected.

25

Consensus pattern: L-C-C-x-[KR]-C-x(4)-[DE]-x-N-x(4)-C-x-C-R-V-P-

Consensus pattern: C-x-H-C-G-C-[KRH]-G-C-[SA]-

[1] McGrew L.L., Dworkin-Rastl E., Dworkin M.B., Richter J.D. *Genes Dev.* 3:803-

- 30 815(1989).

212. G-protein alpha subunit

G proteins couple receptors of extracellular signals to intracellular signaling pathways. The G protein alpha subunit binds guanyl nucleotide and is a weak GTPase. Number of members: 195

- 5 [1] Coleman DE, Berghuis AM, Lee E, Linder ME, Gilman AG, Sprang SR, Science 1994;265:1405-1412.
 [2] How G proteins work: a continuing story. Coleman DE, Sprang SR, Trends Biochem Sci 1996;21:41-44.

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213. Glucose-6-phosphate dehydrogenase active site (G6PD)

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (G6PD) [1] catalyzes the first step in the pentose pathway, the reduction of glucose-6-phosphate to gluconolactone 6-phosphate. A lysine residue has been identified as are active nucleophile associated with the activity of the enzyme. The sequence around this lysine is totally conserved from bacterial to mammalian G6PD's and can be used as a signature pattern

Consensus pattern: D-H-Y-L-G-K-[EQK] [K is the active site residue]-

- 20 [1] Jeffery J., Persson B., Wood I., Bergman T., Jeffery R., Joernvall H. Eur. J. Biochem. 212:41-49(1993).

214. GATA-type zinc finger domain

- 25 The GATA family of transcription factors are proteins that bind to DNA sites with the consensus sequence (A/T)GATA(A/G), found within the regulatory region of a number of genes. Proteins currently known to belong to this family are: - GATA-1 [1] (also known as Eryf1, GF-1 or NF-E1), which binds to the GATA region of globin genes and other genes expressed in erythroid cells. It is a transcriptional activator which probably serves as a
 30 general 'switch' factor for erythroid development. - GATA-2 [2], a transcriptional activator which regulates endothelin-1 gene expression in endothelial cells. - GATA-3 [3], a transcriptional activator which binds to the enhancer of the T-cell receptor alpha and delta genes. - GATA-4 [4], a transcriptional activator expressed in endodermally derived tissues and heart. - Drosophila protein panner (or DGATAa) (gene pnr) which acts as a repressor of

the achaete-scute complex (as-c). - *Bombyx mori* BCFI [5], which regulates the expression of chorion genes. - *Caenorhabditis elegans* elt-1 and elt-2, transcriptional activators of genes containing the GATA region, including vitellogenin genes [6]. - *Ustilago maydis* urbs1 [7], a protein involved in the repression of the biosynthesis of siderophores. - Fission yeast protein

5 GAF2. All these transcription factors contain a pair of highly similar 'zinc finger' type domains with the consensus sequence C-x2-C-x17-C-x2-C. Some other proteins contain a single zinc finger motif highly related to those of the GATA transcription factors. These proteins are: - *Drosophila* box A-binding factor (ABF) (also known as protein serpent (gene *srp*)) which may function as a transcriptional activator protein and may play a key role in the

10 organogenesis of the fat body. - *Emericella nidulans* areA [8], a transcriptional activator which mediates nitrogen metabolite repression. - *Neurospora crassa* nit-2 [9], a transcriptional activator which turns on the expression of genes coding for enzymes required for the use of a variety of secondary nitrogen sources, during conditions of nitrogen limitation. - *Neurospora crassa* white collar proteins 1 and 2 (WC-1 and WC-2), which

15 control expression of light-regulated genes. - *Saccharomyces cerevisiae* DAL81 (or UGA43), a negative nitrogen regulatory protein. - *Saccharomyces cerevisiae* GLN3, a positive nitrogen regulatory protein. - *Saccharomyces cerevisiae* GAT1. - *Saccharomyces cerevisiae* GZF3.

Consensus pattern: C-x-[DN]-C-x(4,5)-[ST]-x(2)-W-[HR]-[RK]-x(3)-[GN]-x(3,4)-C-N-
20 [AS]-C [The four C's are zinc ligands]

[1] Trainor C.D., Evans T., Felsenfeld G., Boguski M.S. *Nature* 343:92-96(1990).

[2] Lee M.E., Temizer D.T., Clifford J.A., Quertermous T. *J. Biol. Chem.* 266:16188-16192(1991).

25 [3] Ho I.-C., Vorhees P., Marin N., Oakley B.K., Tsai S.-F., Orkin S.H., Leiden J.M. *EMBO J.* 10:1187-1192(1991).

[4] Spieth J., Shim Y.H., Lea K., Conrad R., Blumenthal T. *Mol. Cell. Biol.* 11:4651-4659(1991).

[5] Drevet J.R., Skeiky Y.A., Iatrou K. *J. Biol. Chem.* 269:10660-10667(1994).

30 [6] Hawkins M.G., McGhee J.D. *J. Biol. Chem.* 270:14666-14671(1995).

[7] Voisard C.P.O., Wang J., Xu P., Leong S.A., McEvoy J.L. *Mol. Cell. Biol.* 13:7091-7100(1993).

[8] Arst H.N. Jr., Kudla B., Martinez-Rossi N.M., Caddick M.X., Sibley S., Davies R.W. *Trends Genet.* 5:291-291(1989).

[9] Fu Y.-H., Marzluf G.A. Mol. Cell. Biol. 10:1056-1065(1990).

215. Glutamine amidotransferases class-I active site (GATase)

- 5 A large group of biosynthetic enzymes are able to catalyze the removal of the ammonia group from glutamine and then to transfer this group to a substrate to form a new carbon-nitrogen group. This catalytic activity is known as glutamine amidotransferase (GATase) (EC 2.4.2.-) [1]. The GATase domain exists either as a separate polypeptidic subunit or as part of a larger polypeptide fused in different ways to a synthase domain. On the basis of sequence
- 10 similarities two classes of GATase domains have been identified [2,3]: class-I (also known as trpG-type) and class-II (also known as purF-type). Class-I GATase domains have been found in the following enzymes: - The second component of anthranilate synthase (AS) (EC 4.1.3.27) [4]. AS catalyzes the biosynthesis of anthranilate from chorismate and glutamine. AS is generally a dimeric enzyme: the first component can synthesize anthranilate using
- 15 ammonia rather than glutamine, whereas component II provides the GATase activity. In some bacteria and in fungi the GATase component of AS is part of a multifunctional protein that also catalyzes other steps of the biosynthesis of tryptophan. - The second component of 4-amino-4-deoxychorismate (ADC) synthase (EC 4.1.3. -), a dimeric prokaryotic enzyme that function in the pathway that catalyzes the biosynthesis of para-aminobenzoate (PABA) from
- 20 chorismate and glutamine. The second component (gene *pabA*) provides the GATase activity [4]. - CTP synthase (EC 6.3.4.2). CTP synthase catalyzes the final reaction in the biosynthesis of pyrimidine, the ATP-dependent formation of CTP from UTP and glutamine. CTP synthase is a single chain enzyme that contains two distinct domains; the GATase domain is in the C-terminal section [2]. - GMP synthase (glutamine-hydrolyzing) (EC 6.3.5.2).
- 25 GMP synthase catalyzes the ATP-dependent formation of GMP from xanthosine 5'-phosphate and glutamine. GMP synthase is a single chain enzyme that contains two distinct domains; the GATase domain is in the N-terminal section [5]. - Glutamine-dependent carbamoyl-phosphate synthase (EC 6.3.5.5) (GD-CPSase); an enzyme involved in both arginine and pyrimidine biosynthesis and which catalyzes the ATP-dependent formation of
- 30 carbamoyl phosphate from glutamine and carbon dioxide. In bacteria GD-CPSase is composed of two subunits: the large chain (gene *carB*) provides the CPSase activity, while the small chain (gene *carA*) provides the GATase activity. In yeast the enzyme involved in arginine biosynthesis is also composed of two subunits: CPA1 (GATase), and CPA2 (CPSase). In most eukaryotes, the first three steps of pyrimidine biosynthesis are catalyzed by

a large multifunctional enzyme (called URA2 in yeast, rudimentary in *Drosophila*, and CAD in mammals). The GATase domain is located at the N-terminal extremity of this polyprotein [6]. - Phosphoribosylformylglycinamide synthase II (EC 6.3.5.3), an enzyme that catalyzes the fourth step in the de novo biosynthesis of purines. In some species of bacteria, FGAM synthase II is composed of two subunits: a small chain (gene *purQ*) which provides the GATase activity and a large chain (gene *purL*) which provides the amidotransferase activity. - The histidine amidotransferase *hisH*, an enzyme that catalyzes the fifth step in the biosynthesis of histidine in prokaryotes. In the second component of AS a cysteine has been shown [7] to be essential for the amidotransferase activity. The sequence around this residue is well conserved in all the above GATase domains and can be used as a signature pattern for class-I GATase.-

Consensus pattern: [PAS]-[LIVMFYT]-[LIVMFY]-G-[LIVMFY]-C-[LIVMFYN]-G-x-
[QEH]-x-[LIVMFA] [C is the active site residue]-

- [1] Buchanan J.M. Adv. Enzymol. 39:91-183(1973).
- [2] Weng M., Zalkin H. J. Bacteriol. 169:3023-3028(1987).
- [3] Nyunoya H., Lusty C.J. J. Biol. Chem. 259:9790-9798(1984).
- [4] Crawford I.P. Annu. Rev. Microbiol. 43:567-600(1989).
- [5] Zalkin H., Argos P., Narayana S.V.L., Tiedeman A.A., Smith J.M. J. Biol. Chem. 260:3350-3354(1985).
- [6] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B. BioEssays 15:157-164(1993).
- [7] Tso J.Y., Hermodson M.A., Zalkin H. J. Biol. Chem. 255:1451-1457(1980).

216. Glutamine amidotransferases class-II active site (GATase_2)

A large group of biosynthetic enzymes are able to catalyze the removal of the ammonia group from glutamine and then to transfer this group to a substrate to form a new carbon-nitrogen group. This catalytic activity is known as glutamine amidotransferase (GATase) (EC 2.4.2.-)

[1]. The GATase domain exists either as a separate polypeptidic subunit or as part of a larger polypeptide fused in different ways to a synthase domain. On the basis of sequence similarities two classes of GATase domains have been identified [2,3]: class-I(also known as *trpG*-type) and class-II (also known as *purF*-type). Class-II GATase domains have been found in the following enzymes: - Amido phosphoribosyltransferase (glutamine

phosphoribosylpyrophosphate amidotransferase) (EC 2.4.2.14). An enzyme which catalyzes the first step in purine biosynthesis, the transfer of the ammonia group of glutamine to PRPP to form 5-phosphoribosylamine (gene *purF* in bacteria, *ADE4* in yeast). - Glucosamine--fructose-6-phosphate aminotransferase (EC 2.6.1.16). This enzyme catalyzes a key reaction in amino sugar synthesis, the formation of glucosamine 6-phosphate from fructose 6-phosphate and glutamine (gene *glmS* in *Escherichia coli*, *nodM* in *Rhizobium*, *GFA1* in yeast) - Asparagine synthetase (glutamine-hydrolyzing) (EC 6.3.5.4). This enzyme is responsible for the synthesis of asparagine from aspartate and glutamine. A cysteine is present at the N-terminal extremity of the mature form of all these enzymes. The cysteine has been shown, in amido phosphoribosyltransferase [4] and in asparagine synthetase [5] to be important for the catalytic mechanism.

Consensus pattern: <x(0,11)-C-[GS]-[IV]-[LIVMFYW]-[AG] [C is the active site residue]-

- [1] Buchanan J.M. Adv. Enzymol. 39:91-183(1973).
- [2] Weng M., Zalkin H. J. Bacteriol. 169:3023-3028(1987).
- [3] Nyunoya H., Lusty C.J. J. Biol. Chem. 259:9790-9798(1984).
- [4] van Heeke G., Schuster M. J. Biol. Chem. 264:5503-5509(1989).
- [5] Vollmer S.J., Switzer R.L., Hermodson M.A., Bower S.G., Zalkin H. J. Biol. Chem. 258:10582-10585(1983).

217. GDP dissociation inhibitor (GDI)

- [1] Schalk I, Zeng K, Wu SK, Stura EA, Matteson J, Huang M, Tandon A, Wilson IA, Balch WE, Nature 1996;381:42-48.

218. Oxidoreductase family (GFO_IDH_MocA)

This family of enzymes utilise NADP or NAD. This family: is called the

GFO/IDH/MOCA family in swiss-prot.

- [1] Kingston RL, Scopes RK, Baker EN, Structure 1996;4:1413-1428.

219. GHMP kinases putative ATP-binding domain

The following kinases contains, in their N-terminal section, a conserved Gly/Ser-rich region which is probably involved in the binding of ATP [1]. These kinases are listed below. -

Galactokinase (EC 2.7.1.6). - Homoserine kinase (EC 2.7.1.39). - Mevalonate kinase (EC 2.7.1.36). - Phosphomevalonate kinase (EC 2.7.4.2). This group of kinases was called

- 5 'GHMP' (from the first letter of their substrate)

Consensus pattern: [LIVM]-[PK]-x-[GSTA]-x(0,1)-G-L-[GS]-S-S-[GSA]-[GSTAC]-

[1] Tsay Y.H., Robinson G.W. Mol. Cell. Biol. 11:620-631(1991).

10

220. Glucose inhibited division protein A family signatures (GIDA)

Bacterial glucose inhibited division protein A (gene gidA) is a protein of 70Kd whose function is not yet known and whose sequence is highly conserved. It is evolutionary related
15 to yeast hypothetical protein YGL236C, Caenorhabditis elegans hypothetical protein F52H3.2 and a Bacillus subtilis protein called gid (and which is different from B.subtilis gidA). Two highly conserved regions were selected as signature patterns. Both regions are located in the central region of the protein.

- 20 Consensus pattern: [GS]-[PT]-x-Y-C-P-S-[LIVM]-E-x-K-[LIVM]-x-[KR]-

Consensus pattern: A-G-Q-x-[NT]-G-x(2)-G-Y-x-E-[SAG](3)-[QS]-G-[LIVM](2)-A-G-[LIVMT]-N-A-

- 25 221. (GLFV_dehydrog)

Glu / Leu / Phe / Val dehydrogenases active site

- Glutamate dehydrogenases (EC 1.4.1.2, EC 1.4.1.3, and EC 1.4.1.4) (GluDH)
30 are enzymes that catalyze the NAD- or NADP-dependent reversible deamination of glutamate into alpha-ketoglutarate [1,2]. GluDH isozymes are generally involved with either ammonia assimilation or glutamate catabolism.

- Leucine dehydrogenase (EC 1.4.1.9) (LeuDH) is a NAD-dependent enzyme that catalyzes the reversible deamination of leucine and several other aliphatic amino acids to their keto analogues [3].

- Phenylalanine dehydrogenase (EC 1.4.1.20) (PheDH) is a NAD-dependent enzyme that catalyzes the reversible deamidation of L-phenylalanine into phenylpyruvate [4].

- 5 - Valine dehydrogenase (EC 1.4.1.8) (ValDH) is a NADP-dependent enzyme that catalyzes the reversible deamidation of L-valine into 3-methyl-2-oxobutanoate [5].

10 These dehydrogenases are structurally and functionally related. A conserved lysine residue located in a glycine-rich region has been implicated in the catalytic mechanism. The conservation of the region around this residue allows the derivation of a signature pattern for such type of enzymes.

15 Consensus pattern[LIV]-x(2)-G-G-[SAG]-K-x-[GV]-x(3)-[DNST]-[PL] [K is the active site residue] Sequences known to belong to this class detected by the pattern ALL.

20 Note all known sequences from this family have Pro in the last position of the pattern with the exception of yeast GluDH which as Leu.

- 25 [1] Britton K.L., Baker P.J., Rice D.W., Stillman T.J. Eur. J. Biochem. 209:851-859(1992).
 [2] Benachenhou-Lahfa N., Forterre P., Labedan B. J. Mol. Evol. 36:335-346(1993).
 [3] Nagata S., Tanizawa K., Esaki N., Sakamoto Y., Ohshima T., Tanaka H., Soda K. Biochemistry 27:9056-9062(1988).
 [4] Takada H., Yoshimura T., Ohshima T., Esaki N., Soda K. J. Biochem. 109:371-376(1991).
 [5] Hutchinson C.R., Tang L. J. Bacteriol. 175:4176-4185(1993).

222. GMC oxidoreductases signatures

- 30 The following FAD flavoproteins oxidoreductases have been found [1,2] to be evolutionary related. These enzymes, which are called 'GMC oxidoreductases', are listed below. - Glucose oxidase (EC 1.1.3.4) (GOX) from *Aspergillus niger*. Reaction catalyzed: glucose + oxygen -> delta-gluconolactone + hydrogen peroxide. - Methanol oxidase (EC 1.1.3.13) (MOX) from fungi. Reaction catalyzed: methanol + oxygen -> acetaldehyde + hydrogen peroxide. - Choline dehydrogenase (EC 1.1.99.1) (CHD) from bacteria. Reaction catalyzed: choline +

unknown acceptor -> betaine acetaldehyde + reduced acceptor. - Glucose dehydrogenase (GLD) (EC 1.1.99.10) from *Drosophila*. Reaction catalyzed: glucose + unknown acceptor -> delta-gluconolactone + reduced acceptor. - Cholesterol oxidase (CHOD) (EC 1.1.3.6) from *Brevibacterium sterolicum* and *Streptomyces* strain SA-COO. Reaction catalyzed: cholesterol + oxygen -> cholest-4-en-3-one + hydrogen peroxide. - AlkJ [3], an alcohol dehydrogenase from *Pseudomonas oleovorans*, which converts aliphatic medium-chain-length alcohols into aldehydes. This family also includes a lyase: - (R)-mandelonitrile lyase (EC 4.1.2.10) (hydroxynitrile lyase) from plants [4], an enzyme involved in cyanogenesis, the release of hydrogen cyanide from injured tissues. These enzymes are proteins of size ranging from 556 (CHD) to 664 (MOX) amino acid residues which share a number of regions of sequence similarities. One of these regions, located in the N-terminal section, corresponds to the FAD ADP-binding domain. The function of the other conserved domains is not yet known; two of these domains were selected as signature patterns. The first one is located in the N-terminal section of these enzymes, about 50 residues after the ADP-binding domain, while the second one is located in the central section.

Consensus pattern: [GA]-[RKN]-x-[LIV]-G(2)-[GST](2)-x-[LIVM]-N-x(3)-[FYWA]-x(2)-[PAG]-x(5)-[DNESH]-

Consensus pattern: [GS]-[PSTA]-x(2)-[ST]-P-x-[LIVM](2)-x(2)-S-G-[LIVM]-G-

[1] Cavener D.R. J. Mol. Biol. 223:811-814(1992).

[2] Henikoff S., Henikoff J.G. Genomics 19:97-107(1994).

[3] van Beilen J.B., Eggink G., Enequist H., Bos R., Witholt B. Mol. Microbiol. 6:3121-3136(1992).

[4] Cheng I.P., Poulton J.E. Plant Cell Physiol. 34:1139-1143(1993).

223. (GMP_synt_C)

Glutamine amidotransferases class-I active site

A large group of biosynthetic enzymes are able to catalyze the removal of the ammonia group from glutamine and then to transfer this group to a substrate to form a new carbon-nitrogen group. This catalytic activity is known as glutamine amidotransferase (GATase) (EC 2.4.2.-) [1]. The GATase domain exists either as a separate polypeptidic subunit or as part of a larger

polypeptide fused in different ways to a synthase domain. On the basis of sequence similarities two classes of GATase domains have been identified [2,3]: class-I (also known as trpG-type) and class-II (also known as purF-type). Class-I GATase domains have been found in the following enzymes:

5 - The second component of anthranilate synthase (AS) (EC 4.1.3.27) [4]. AS catalyzes the biosynthesis of anthranilate from chorismate and glutamine. AS is generally a dimeric enzyme: the first component can synthesize anthranilate using ammonia rather than glutamine, whereas component II provides the GATase activity. In some bacteria and in fungi
10 the GATase component of AS is part of a multifunctional protein that also catalyzes other steps of the biosynthesis of tryptophan.

- The second component of 4-amino-4-deoxychorismate (ADC) synthase (EC 4.1.3. -), a dimeric prokaryotic enzyme that function in the pathway that catalyzes the biosynthesis of para-aminobenzoate (PABA) from chorismate and glutamine. The second component (gene
15 pabA) provides the GATase activity [4].

- CTP synthase (EC 6.3.4.2). CTP synthase catalyzes the final reaction in the biosynthesis of pyrimidine, the ATP-dependent formation of CTP from UTP and glutamine. CTP synthase is a single chain enzyme that contains two distinct domains; the GATase domain is in the C-terminal section [2].

20 - GMP synthase (glutamine-hydrolyzing) (EC 6.3.5.2). GMP synthase catalyzes the ATP-dependent formation of GMP from xanthosine 5'-phosphate and glutamine. GMP synthase is a single chain enzyme that contains two distinct domains; the GATase domain is in the N-terminal section [5].

- Glutamine-dependent carbamoyl-phosphate synthase (EC 6.3.5.5) (GD-CPSase); an
25 enzyme involved in both arginine and pyrimidine biosynthesis and which catalyzes the ATP-dependent formation of carbamoyl phosphate from glutamine and carbon dioxide. In bacteria GD-CPSase is composed of two subunits: the large chain (gene carB) provides the CPSase activity, while the small chain (gene carA) provides the GATase activity. In yeast the enzyme involved in arginine biosynthesis is also composed of two subunits: CPA1 (GATase),
30 and CPA2 (CPSase). In most eukaryotes, the first three steps of pyrimidine biosynthesis are catalyzed by a large multifunctional enzyme (called URA2 in yeast, rudimentary in Drosophila, and CAD in mammals). The GATase domain is located at the N-terminal extremity of this polypeptide [6].

- Phosphoribosylformylglycinamide synthase II (EC 6.3.5.3), an enzyme that catalyzes the fourth step in the de novo biosynthesis of purines. In some species of bacteria, FGAM synthase II is composed of two subunits: a small chain (gene purQ) which provides the GATase activity and a large chain (gene purL) which provides the aminator activity.

5 - The histidine amidotransferase hisH, an enzyme that catalyzes the fifth step in the biosynthesis of histidine in prokaryotes.

In the second component of AS a cysteine has been shown [7] to be essential for the amidotransferase activity. The sequence around this residue is well conserved in all the
10 above GATase domains and can be used as a signature pattern for class-I GATase.

Consensus pattern[PAS]-[LIVMFY*]-[LIVMFY]-G-[LIVMFY]-C-[LIVMFYN]-G-x-
[QEH]-x-[LIVMFA] [C is the active site residue] Sequences known to belong to this class
detected by the pattern ALL, except for 6 sequences.

15 Note: in the first position of the pattern Pro is found in all cases except in the slime mold GD-CPSase where it is replaced by Ala.

[1] Buchanan J.M. Adv. Enzymol. 39:91-183(1973).

20 [2] Weng M., Zalkin H. J. Bacteriol. 169:3023-3028(1987).

[3] Nyunoya H., Lusty C.J. J. Biol. Chem. 259:9790-9798(1984).

[4] Crawford I.P. Annu. Rev. Microbiol. 43:567-600(1989).

[5] Zalkin H., Argos P., Narayana S.V.L., Tiedeman A.A., Smith J.M. J. Biol. Chem. 260:3350-3354(1985).

25 [6] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B. BioEssays 15:157-164(1993).

[7] Tso J.Y., Hermodson M.A., Zalkin H. J. Biol. Chem. 255:1451-1457(1980).

30 224. Glutathione peroxidases signatures (GSHPx)

Glutathione peroxidase (EC 1.11.1.9) (GSHPx) [1,2] is an enzyme that catalyzes the reduction of hydroxyperoxides by glutathione. Its main function is to protect against the damaging effect of endogenously formed hydroxyperoxides. In higher vertebrates at least four forms of GSHPx are known to exist: a ubiquitous cytosolic form (GSHPx-1), a

gastrointestinal cytosolic for (GSHPx-GI) [3], a plasma secreted form (GSHPx-P) [4], and a epididymal secretory form (GSHPx-EP). In addition to these characterized forms, the sequence of a protein of unknown function [5] has been shown to be evolutionary related to those of GSHPx's. In filarial nematode parasites such as *Brugia pahangi* the major soluble cuticular protein, known as gp29, is a secreted GSHPx which could provide a mechanism of resistance to the immune reaction of the mammalian host by neutralizing the products of the oxidative burst of leukocytes [6]. *Escherichia coli* protein btuE, a periplasmic protein involved in the transport of vitamin B12, is also evolutionary related to GSHPx's; the significance of this relationship is not yet clear. Selenium, in the form of selenocysteine [7] is part of the catalytic site of GSHPx. The sequence around the selenocysteine residue is moderately well conserved in GSHPx's and the related proteins and can be used as a signature pattern. As a second signature for this family of proteins a highly conserved octapeptide located in the central section of these proteins was selected.

15 Consensus pattern: [GN]-[RKHNFCY]-x-[LIVMFC]-[LIVMF](2)-x-N-[VT]-x-[STC]-x-C-[GA]-x-T [C is the active site selenocysteine residue]

Consensus pattern: [LIV]-[AGD]-F-P-[CS]-[NG]-Q-

[1] Mannervik B. Meth. Enzymol. 113:490-495(1985).

20 [2] Mullenbach G.T., Tabrizi A., Irvine B.D., Bell G.I., Tainer J.A., Hallewell R.A. Protein Eng. 2:239-246(1988).

[3] Chu F.F., Doroshov J.H., Esworthy R.S. J. Biol. Chem. 268:2571-2576(1993).

[4] Takahashi K., Akasaka M., Yamamoto Y., Kobayashi C., Mizoguchi J., Koyama J. J. Biochem. 108:145-148(1990).

25 [5] Dunn D.K., Howells D.D., Richardson J., Goldfarb P.S. Nucleic Acids Res. 17:6390-6390(1989).

[6] Cookson E., Blaxter M.L., Selkirk M.E. Proc. Natl. Acad. Sci. U.S.A. 89:5837-5841(1992).

[7] Stadtman T.C. Annu. Rev. Biochem. 59:111-127(1990).

30

225. (GST)

Glutathione S-transferases

Function: conjugation of reduced glutathione to a variety of targets. Also included in the alignment, but are not GSTs S-crystallins from squid. Similarity to GST was previously noted. Eukaryotic elongation factors 1-gamma. Not known to have GST activity; similarity not previously recognized. Supported by HMM and manual alignment inspection. HSP26 family of stress-related proteins. including auxin-regulated proteins in plants and stringent starvation proteins in *E. coli*. Not known to have GST activity. Similarity not previously recognized. Supported by HMM and manual alignment inspection. Alignment spans entire protein.

226. GTP1/OBG family signature

A widespread family of GTP-binding proteins has been recently characterized [1,2]. This family currently includes: - Mouse and *Xenopus* protein DRG. - Human protein DRG2. - *Drosophila* protein 128up. - Fission yeast protein gtp1. - *A. Halobacterium cutirubrum* hypothetical protein in a ribosomal protein gene cluster. - *Bacillus subtilis* protein obg. Obg has been experimentally shown to bind GTP. - *Escherichia coli* hypothetical protein yhbZ. - *Haemophilus influenzae* hypothetical protein HI0877. - *Mycoplasma genitalium* hypothetical protein MG384. - Yeast hypothetical protein YAL036c (FUN11). - Yeast hypothetical protein YGR173w. - *Caenorhabditis elegans* hypothetical protein C02F5.3. The function of the proteins that belong to this family is not yet known. They are polypeptides of about 40 to 48 Kd which contain the five small sequence elements characteristic of GTP-binding proteins [3]. As a signature pattern the region that correspond to the ATP/GTP B motif (also called G-3 in GTP-binding proteins) was selected.

Consensus pattern: D-[LIVM]-P-G-[LIVM](2)-[DEY]-[GN]-A-x(2)-G-x-G -

[1] Sazuka T., Tomooka Y., Ikawa Y., Noda M., Kumar S. *Biochem. Biophys. Res. Commun.* 189:363-370(1992).

[2] Hudson J.D., Young P.G. *Gene* 125:191-193(1993).

[3] Bourne H.R., Sanders D.A., McCormick F. *Nature* 349:117-127(1991).

227. (GTP_EFTU1)

ATP/GTP-binding site motif A (P-loop)

From sequence comparisons and crystallographic data analysis it has been shown [1,2,3,4,5,6] that an appreciable proportion of proteins that bind ATP or GTP share a number of more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region, which typically forms a flexible loop between a beta-strand and an alpha-helix.

5 This loop interacts with one of the phosphate groups of the nucleotide. This sequence motif is generally referred to as the 'A' consensus sequence [1] or the 'P-loop' [5]. There are numerous ATP- or GTP-binding proteins in which the P-loop is found. Listed below are a number of protein families for which the relevance of the presence of such motif has been noted: - ATP synthase alpha and beta subunits (see <PDOC00137>). - Myosin heavy chains. - Kinesin
10 heavy chains and kinesin-like proteins (see <PDOC00343>). - Dynamins and dynamin-like proteins (see <PDOC00362>). - Guanylate kinase (see <PDOC00670>). - Thymidine kinase (see <PDOC00524>). - Thymidylate kinase (see <PDOC01034>). - Shikimate kinase (see <PDOC00868>). - Nitrogenase iron protein family (nifH/frxC) (see <PDOC00580>). - ATP-binding proteins involved in 'active transport' (ABC transporters) [7] (see <PDOC00185>). -
15 DNA and RNA helicases [8,9,10]. - GTP-binding elongation factors (EF-Tu, EF-1alpha, EF-G, EF-2, etc.). - Ras family of GTP-binding proteins (Ras, Rho, Rab, Ral, Ypt1, SEC4, etc.). - Nuclear protein ran (see <PDOC00859>). - ADP-ribosylation factors family (see <PDOC00781>). - Bacterial dnaA protein (see <PDOC00771>). - Bacterial recA protein (see <PDOC00131>). - Bacterial recF protein (see <PDOC00539>). - Guanine nucleotide-binding
20 proteins alpha subunits (Gi, Gs, Gt, G0, etc.). - DNA mismatch repair proteins mutS family (see <PDOC00388>). - Bacterial type II secretion system protein E (see <PDOC00567>). Not all ATP- or GTP-binding proteins are picked-up by this motif. A number of proteins escape detection because the structure of their ATP-binding site is completely different from that of the P-loop. Examples of such proteins are the E1-E2 ATPases or the glycolytic kinases. In
25 other ATP- or GTP-binding proteins the flexible loop exists in a slightly different form; this is the case for tubulins or protein kinases. A special mention must be reserved for adenylate kinase, in which there is a single deviation from the P-loop pattern: in the last position Gly is found instead of Ser or Thr.

30 -Consensus pattern: [AG]-x(4)-G-K-[ST]-

[1] Walker J.E., Saraste M., Runswick M.J., Gay N.J. EMBO J. 1:945-951(1982).

[2] Moller W., Amons R. FEBS Lett. 186:1-7(1985).

[3] Fry D.C., Kuby S.A., Mildvan A.S. Proc. Natl. Acad. Sci. U.S.A. 83:907-911(1986).

- [4] Dever T.E., Glynias M.J., Merrick W.C. Proc. Natl. Acad. Sci. U.S.A. 84:1814-1818(1987).
- [5] Saraste M., Sibbald P.R., Wittinghofer A. Trends Biochem. Sci. 15:430-434(1990).
- [6] Koonin E.V. J. Mol. Biol. 229:1165-1174(1993).
- 5 [7] Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P. J. Bioenerg. Biomembr. 22:571-592(1990).
- [8] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).
- [9] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).
- 10 [10] Gorbalenya A.E., Koonin E.V., Donchenko A.P., Blinov V.M. Nucleic Acids Res. 17:4713-4730(1989).

GTP-binding elongation factors signature (GTP_EFTU2)

Elongation factors [1,2] are proteins catalyzing the elongation of peptide chains in protein biosynthesis. In both prokaryotes and eukaryotes, there are three distinct types of elongation factors, as described in the following table: -----

-----	Eukaryotes	Prokaryotes	Function	-----
-----	EF-1alpha	EF-Tu	Binds GTP and an aminoacyl-tRNA; delivers the latter to the A site of ribosomes. EF-1beta EF-Ts Interacts with EF-1a/EF-Tu to displace GDP and thus allows the regeneration of GTP-EF-1a. EF-2 EF-G Binds GTP and peptidyl-tRNA and translocates the latter from the A site to the P site. -----	-----
-----	The GTP-binding elongation factor family also includes the following proteins: - Eukaryotic peptide chain release factor GTP-binding subunits [3]. These proteins interact with release factors that bind to ribosomes that have encountered a stop codon at their decoding site and help them to induce release of the nascent polypeptide. The yeast protein was known as SUP2 (and also as SUP35, SUF12 or GST1) and the human homolog as GST1-Hs. - Prokaryotic peptide chain release factor 3 (RF-3) (gene prfC). RF-3 is a class-II RF, a GTP-binding protein that interacts with class I RFs (see <PDOC00607>) and enhance their activity [4]. - Prokaryotic GTP-binding protein lepA and its homolog in yeast (gene GUF1) and in Caenorhabditis elegans (ZK1236.1). - Yeast HBS1 [5]. - Rat statin S1 [6], a protein of unknown function which is highly similar to EF-1alpha. - Prokaryotic selenocysteine-specific elongation factor selB [7], which seems to replace EF-Tu for the insertion of selenocysteine directed by the UGA codon. - The tetracycline resistance proteins tetM/tetO [8,9] from various bacteria such as Campylobacter jejuni, Enterococcus faecalis,			

----- EF-1alpha EF-Tu Binds GTP and an aminoacyl-tRNA; delivers the latter to the A site of ribosomes. EF-1beta EF-Ts Interacts with EF-1a/EF-Tu to displace GDP and thus allows the regeneration of GTP-EF-1a. EF-2 EF-G Binds GTP and peptidyl-tRNA and translocates the latter from the A site to the P site. -----

-----The GTP-binding elongation factor family also includes the following proteins: - Eukaryotic peptide chain release factor GTP-binding subunits [3]. These proteins interact with release factors that bind to ribosomes that have encountered a stop codon at their decoding site and help them to induce release of the nascent polypeptide. The yeast protein was known as SUP2 (and also as SUP35, SUF12 or GST1) and the human homolog as GST1-Hs. - Prokaryotic peptide chain release factor 3 (RF-3) (gene prfC). RF-3 is a class-II RF, a GTP-binding protein that interacts with class I RFs (see <PDOC00607>) and enhance their activity [4]. - Prokaryotic GTP-binding protein lepA and its homolog in yeast (gene GUF1) and in Caenorhabditis elegans (ZK1236.1). - Yeast HBS1 [5]. - Rat statin S1 [6], a protein of unknown function which is highly similar to EF-1alpha. - Prokaryotic selenocysteine-specific elongation factor selB [7], which seems to replace EF-Tu for the insertion of selenocysteine directed by the UGA codon. - The tetracycline resistance proteins tetM/tetO [8,9] from various bacteria such as Campylobacter jejuni, Enterococcus faecalis,

Streptococcus mutans and Ureaplasma urealyticum. Tetracycline binds to the prokaryotic ribosomal 30S subunit and inhibits binding of aminoacyl-tRNAs. These proteins abolish the inhibitory effect of tetracycline on protein synthesis. - Rhizobium nodulation protein nodQ [10]. - Escherichia coli hypothetical protein yihK [11]. In EF-1-alpha, a specific region has been shown [12] to be involved in a conformational change mediated by the hydrolysis of GTP to GDP. This region is conserved in both EF-1alpha/EF-Tu as well as EF-2/EF-G and thus seems typical for GTP-dependent proteins which bind non-initiator tRNAs to the ribosome. The pattern developed for this family of proteins include that conserved region.

- 10 Consensus pattern: D-[KRSTGANQFYW]-x(3)-E-[KRAQ]-x-[RKQD]-[GC]-[IVMK]-[ST]-[IV]-x(2)-[GSTACKRNQ]-

[1] Concise Encyclopedia Biochemistry, Second Edition, Walter de Gruyter, Berlin New-York (1988).

- 15 [2] Moldave K. Annu. Rev. Biochem. 54:1109-1149(1985).

[3] Stansfield I., Jones K.M., Kushnirov V.V., Dagkesamanskaya A.R., Poznyakovski A.I., Paushkin S.V., Nierras C.R., Cox B.S., Ter-Avanesyan M.D., Tuite M.F. EMBO J. 14:4365-4373(1995).

[4] Grentzmann G., Brechemier-Baey D., Heurgue-Hamard V., Buckingham R.H. J. Biol. Chem. 270:10595-10600(1995).

- 20 [5] Nelson R.J., Ziegelhoffer T., Nicolet C., Werner-Washburne M., Craig E.A. Cell 71:97-105(1992).

[6] Ann D.K., Moutsatsos I.K., Nakamura T., Lin H.H., Mao P.-L., Lee M.-J., Chin S., Liem R.K.H., Wang E. J. Biol. Chem. 266:10429-10437(1991).

- 25 [7] Forchhammer K., Leinfelder W., Bock A. Nature 342:453-456(1989).

[8] Manavathu E.K., Hiratsuka K., Taylor D.E. Gene 62:17-26(1988).

[9] Leblanc D.J., Lee L.N., Titmas B.M., Smith C.J., Tenover F.C. J. Bacteriol. 170:3618-3626(1988).

[10] Cervantes E., Sharma S.B., Maillet F., Vasse J., Truchet G., Rosenberg C. Mol.

- 30 Microbiol. 3:745-755(1989).

[11] Plunkett G. III, Burland V.D., Daniels D.L., Blattner F.R. Nucleic Acids Res. 21:3391-3398(1993).

[12] Moller W., Schipper A., Amons R. Biochimie 69:983-989(1987).

228. GTP cyclohydrolase II.

GTP cyclohydrolase II catalyses the first committed step in the biosynthesis of riboflavin.

- 5 [1] Richter G, Ritz H, Katzenmeier G, Volk R, Kohnle A, Lottspeich F, Allendorf D, Bacher A, J Bacteriol 1993;175:4045-4051.

229. Galactose-1-phosphate uridyl transferase signatures (GalP_UDP_transf)

- 10 Galactose-1-phosphate uridyl transferase (EC 2.7.7.10) (galT) catalyzes the transfer of an uridyldiphosphate group on galactose (or glucose) 1-phosphate. During the reaction, the uridyl moiety links to a histidine residue. In the Escherichia coli enzyme, it has been shown [1] that two histidine residues separated by a single proline residue are essential for enzyme activity. On the basis of sequence similarities, two apparently unrelated families seem to
- 15 exist. Class-I enzymes are found in eukaryotes as well as some bacteria such as Escherichia coli or Streptomyces lividans, while class-II enzymes have been found so far only in bacteria such as Bacillus subtilis or Lactobacillus helveticus [2]. Signature patterns for both families were developed. For class-I enzymes the signature is based on the active site residues. For class-II enzymes a region which also includes two conserved histidines was chosen.

20 Consensus pattern: F-E-N-[RK]-G-x(3)-G-x(4)-H-P-H-x-Q [The two H's are the active site residues]-

Consensus pattern: D-L-P-I-V-G-G-[ST]-[LIVM](2)-[SA]-H-[DEN]-H-[FY]-Q-G-G -

Note: class-I enzymes are structurally related to the HIT family of proteins (see

- 25 <PDOC00694

[1] Reichardt J.K.V., Berg P. Nucleic Acids Res. 16:9017-9026(1988).

[2] Mollet B., Pilloud N. J. Bacteriol. 173:4464-4473(1991).

30 230. Gamma-thionins family signature

The following small plant proteins are evolutionary related:

- Gamma-thionins from wheat endosperm (gamma-purothionins) and barley (gamma- hordothionins) which are toxic to animal cells and inhibit protein synthesis in cell free systems [1].
- A flower-specific thionin (FST) from tobacco [2].
- Antifungal proteins (AFP) from the seeds of Brassicaceae species such as radish, mustard, turnip and *Arabidopsis thaliana* [3].
- Inhibitors of insect alpha-amylases from sorghum [4].
- Probable protease inhibitor P322 from potato.
- A germination-related protein from cowpea [5].
- Anther-specific protein SF18 from sunflower [6]. SF18 is a protein that contains a gamma-thionin domain at its N-terminus and a proline-rich C- terminal domain.
- Soybean sulfur-rich protein SE60 [7].
- *Vicia faba* antibacterial peptides fabatin-1 and -2.

In their mature form, these proteins generally consist of about 45 to 50 amino-acid residues. As shown in the following schematic representation, these peptides contain eight conserved cysteines involved in disulfide bonds.

```

+-----+ +-----+ ||||
xxCxxxxxxxxCxxxxCxxxCxxxxxxxxCxxxxxCxxxxC *****|***||
+---+-----+ +-----+

```

'C': conserved cysteine involved in a disulfide bond.

'*': position of the pattern.

Consensus pattern: [KRG]-x-C-x(3)-[SV]-x(2)-[FYWH]-x-[GF]-x-C-x(5)-C-x(3)-C [The four C's are involved in disulfide bonds]-

- [1] Bruix M., Jimenez M.A., Santoro J., Gonzalez C., Colilla F.J., Mendez E., Rico M. *Biochemistry* 32:715-724(1993).
- [2] Gu Q., Kawata E.E., Morse M.-J., Wu H.-M., Cheung A.Y. *Mol. Gen. Genet.* 234:89-96(1992).
- [3] Terras F.R.G., Torrekens S., van Leuven F., Osborn R.W., Vanderleyden J., Cammue B.P.A., Broekaert W.F. *FEBS Lett.* 316:233-240(1993).
- [4] Bloch C. Jr., Richardson M. *FEBS Lett.* 279:101-104(1991).
- [5] Ishibashi N., Yamauchi D., Minamikawa T. *Plant Mol. Biol.* 15:59-64(1990).
- [7] Choi Y., Choi Y.D., Lee J.S. *Plant Physiol.* 101:699-700(1993).

231. Gelsolin. Gelsolin repeat. Number of members: 170

- 5 [1]Medline: 97433077. The crystal structure of plasma gelsolin: implications for actin severing, capping, and nucleation. Burtnick LD, Koepf EK, Grimes J, Jones EY, Stuart DI, McLaughlin PJ, Robinson RC; Cell 1997;90:661-670.

10 232. Germin family signature

Germins [1] are a family of homopentameric cereal glycoproteins expressed during germination which may play a role in altering the properties of cell walls during germinative growth. It has been shown that wheat and barleygermins act as oxalate oxidases (EC 1.2.3.4), an enzyme that catalyzes the oxidative degradation of oxalate to carbonate and hydrogen peroxide. Germins are highly similar to: - Germin-like proteins from various plants such as rape, violet or white mustard. - Slime mold spherulins 1a and 1b which are proteins that accumulate specifically during spherulation, a process induced by various forms of environmental stress which leads to encystment and dormancy. As a signature pattern the best conserved region was selected: a decapeptide located in the central section of these proteins.

20 Consensus pattern: G-x(4)-H-x-H-P-x-A-x-E-[LIVM]-

[1] Lane B.G. FASEB J. 8:294-301(1994).

25 233. (GlutR)

Glutamyl-tRNA reductase signature

- 30 Delta-aminolevulinic acid (ALA) is the obligatory precursor for the synthesis of all tetrapyrroles including porphyrin derivatives such as chlorophyll and heme. ALA can be synthesized via two different pathways: the Shemin (or C4) pathway which involves the single step condensation of succinyl-CoA and glycine and which is catalyzed by ALA synthase (EC 2.3.1.37) and via the C5 pathway from the five-carbon skeleton of glutamate.

The C5 pathway operates in the chloroplast of plants and algae, in cyanobacteria, in some eubacteria and in archaebacteria.

The initial step in the C5 pathway is carried out by glutamyl-tRNA reductase (GluTR) [1] which catalyzes the NADP-dependent conversion of glutamate- tRNA(Glu) to glutamate-1-semialdehyde (GSA) with the concomitant release of tRNA(Glu) which can then be recharged with glutamate by glutamyl-tRNA synthetase.

GluTR is a protein of about 50 Kd (467 to 550 residues) which contains a few conserved region. The best conserved region is located in positions 99 to 122 in the sequence of known GluTR. This region seems important for the activity of the enzyme. We have developed a signature pattern from that conserved region.

Consensus pattern H-[LIVM]-x(2)-[LIVM]-[GSTAC](3)-[LIVM]-[DEQ]-S-[LIVMA]-[LIVM](2)-[GF]-E-x-[EQR]-[IV]-[LIT]-[STAG]-Q-[LIVM]-[KR] Sequences known to belong to this class detected by the pattern ALL.

[1] Jahn D., Verkamp E., Soell D. Trends Biochem. Sci. 17:215-218(1992).

234. (Glycoprotease)

Glycoprotease family signature (aka Peptidase_M22)

Glycoprotease (GCP) (EC 3.4.24.57) [1], or o-sialoglycoprotein endopeptidase, is a metalloprotease secreted by *Pasteurella haemolytica* which specifically cleaves O-sialoglycoproteins such as glycophorin A. The sequence of GCP is highly similar to the following uncharacterized proteins:

- *Escherichia coli* hypothetical protein ygiD (ORF-X).
- *Bacillus subtilis* hypothetical protein ydiE.
- *Mycobacterium leprae* hypothetical protein U229E.
- *Mycobacterium tuberculosis* hypothetical protein MtCY78.10.
- *Synechocystis* strain PCC 6803 hypothetical protein slr0807.
- *Methanococcus jannaschii* hypothetical protein MJ1130.

- Haloarcula marismortui hypothetical protein in HSH 3' region.
- Yeast hypothetical protein YKR038c.
- Yeast hypothetical protein QRI7.

- 5 One of the conserved regions contains two conserved histidines. It is possible that this region is involved in coordinating a metal ion such as zinc.

Consensus pattern[KR]-[GSAT]-x(4)-[FYWLH]-[DONGK]-x-P-x-[LIVMFY]-x(3)-H- x(2)-[AG]-H-[LIVM] Sequences known to belong to this class detected by the pattern ALL.

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Note: these proteins belong to family M22 in the classification of peptidases [2,E1].

[1] Abdullah K.M., Lo R.Y.C., Mellors A. J. Bacteriol. 173:5597-5603(1991).

15

[2] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

235. (Glucosamine_iso)

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Glucosamine/galactosamine-6-phosphate isomerases signature

Glucosamine-6-phosphate isomerase (EC 5.3.1.10) (or Glc-6-P deaminase) is the enzyme responsible for the conversion of glucosamine 6-phosphate into fructose6 phosphate [1]. It is the last specific step in the pathway for N-acetylglucosamine (GlcNAC) utilization in bacteria such as Escherichia coli (gene nagB) or in fungi such as Candida albicans (gene NAG1).Glc-

25

6-P isomerase is evolutionary related to: - A putative Escherichia coli galactosamine-6-phosphate isomerase (gene agaI) [2]. - Escherichia coli hypothetical protein yieK. - Bacillus subtilis hypothetical protein ybft. As a signature pattern a conserved region located in the central part of these enzymes was selected. This region contains a conserved histidine which has been shown [1], in nagB, to be important for the pyranose ring-opening step of the catalytic mechanism

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Consensus pattern: [LIVM]-x(3)-G-x-[LIT]-x-[LIV]-x-[LIVM]-x-G-[LIVM]-G-x- [DEN]-G-H-

[1] Oliva G., Fontes M.R.M., Garratt R.C., Altamirano M.M., Calcagno M.L., Horjales E. Structure 3:1323-1332(1995).

[2] Reizer J., Ramseier T.M., Reizer A., Charbit A., Saier M.H. Jr. Microbiology 142:231-250(1996).

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236. Pneumovirus attachment glycoprotein G (glycoprotein G)

This family includes attachment proteins from respiratory syncytial virus. Glycoprotein G has not been shown to have any neuraminidase or hemagglutinin activity (Swiss-Prot). The amino terminus is thought to be cytoplasmic, and the carboxyl terminus extracellular. The extracellular region contains four completely conserved cysteine residues.

10

[1] Johnson PR, Spriggs MK, Olmsted RA, Collins PL, Proc Natl Acad Sci U S A 1987;84:5625-5629.

15

237. Glycosyl transferases group 1

Mutations in this domain of Swiss:P37287 lead to disease (Paroxysmal Nocturnal haemoglobinuria). Members of this family transfer activated sugars to a variety of substrates, including glycogen, Fructose-6-phosphate and lipopolysaccharides. Members of this family transfer UDP, ADP, GDP or CMP linked sugars. The eukaryotic glycogen synthases may be distant members of this family.

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238. Glycosyl transferases (Glycos_transf_2)

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Diverse family, transferring sugar from UDP-glucose, UDP-N-acetyl-galactosamine, GDP-mannose or CDP-abequose, to a range of substrates including cellulose, dolichol phosphate and teichoic acids.

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239. (Glucos_transf_3)

Thymidine and pyrimidine-nucleoside phosphorylases signature

Thymidine phosphorylase (EC 2.4.2.4) catalyzes the reversible phosphorolysis of thymidine, deoxyuridine and their analogues to their respective bases and 2-deoxyribose 1-

phosphate. This enzyme regulates the availability of thymidine and is therefore essential to nucleic acid metabolism.

In *Escherichia coli* (gene *deoA*), the enzyme is a dimer of identical subunits of about 48 Kd [1]. In humans it was first identified as platelet-derived endothelial cell growth factor (PD-ECGF) [E1] before being recognized [2] as thymidine phosphorylase.

Bacterial pyrimidine-nucleoside phosphorylase (EC 2.4.2.2) (gene *pdp*) [3] is an enzyme evolutionary and structurally related to thymidine phosphorylase.

A well conserved region of 19 residues located in the N-terminal part of these proteins signature pattern for these enzymes was selected.

Consensus pattern S-[GS]-R-[GA]-[LIV]-x(2)-[TA]-[GA]-G-T-x-D-x-[LIV]-E Sequences known to belong to this class detected by the pattern ALL.

[1] Walter M.R., Cook W.J., Cole L.B., Short S.A., Koszalka G.W., Krenitsky T.A., Ealick S.E. *J. Biol. Chem.* 265:14016-14022(1990).

[2] Furukawa T., Yoshimura A., Sumizawa T., Haraguchi M., Akiyama S.-I., Fukui K., Yamada Y. *Nature* 356:668-668(1992).

[3] Saxild H.H., Andersen L.N., Hammer K. *J. Bacteriol.* 178:424-434(1996).

240. Glycosyl_transf_4. Glycosyl transferase. Number of members: 44.

[1] Medline: 95252686. A family of UDP-GlcNAc/MurNAc: polyisoprenol-P GlcNAc/MurNAc-1-P transferases. Lehrman MA; Glycobiology 1994;4:768-771.

241. Glycosyl hydrolases family 15. 21 members.

242. Glycosyl hydrolases family 16 signature

It has been shown [1] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities: - Bacterial beta-1,3-1,4-glucanases, or lichenases, (EC 3.2.1.73) mainly from *Bacillus* but also from *Clostridium thermocellum* (gene *licB*), *Fibrobacter succinogenes* and *Rhodothermus marinus* (gene *bglA*). - *Bacillus* 5 *circulans* beta-1,3-glucanase A1 (EC 3.2.1.39) (gene *glcA*). - *Lamarinase* (EC 3.2.1.6) from *Clostridium thermocellum* (gene *lam1*). - *Streptomyces coelicolor* agarase (EC 3.2.1.81) (gene *dagA*). - *Alteromonas carrageenovora* kappa-carrageenase (EC 3.2.1.83) (gene *cgkA*). Two closely clustered conserved glutamates have been shown [2] to be involved in the catalytic activity of *Bacillus licheniformis* lichenase. The region was used that contains these 10 residues as a signature pattern.

Consensus pattern: E-[LIV]-D-[LIV]-x(0,1)-E-x(2)-[GQ]-[KRNF]-x-[PSTA] [The two E's are active site residues]-

[1] Henrissat B. *Biochem. J.* 280:309-316(1991).

[2] Juncosa M., Pons J., Dot T., Querol E., Planas A. J. *Biol. Chem.* 269:14530-14535(1994).

243. Glycosyl hydrolases family 17 signature

It has been shown [1,2] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities: - Glucan endo-1,3-beta-glucosidases (EC 3.2.1.39) (endo-(1->3)-beta- glucanase) from various plants. This enzyme may be involved in the defense of plants against pathogens through its ability to degrade fungal cell wall polysaccharides. - Glucan 1,3-beta-glucosidase (EC 3.2.1.58) (exo-(1->3)-beta-glucanase) 25 from yeast (gene *BGL2*). This enzyme may play a role in cell expansion during growth, in cell-cell fusion during mating, and in spore release during sporulation. - Lichenases (EC 3.2.1.73) (endo-(1->3,1->4)-beta-glucanase) from various plants. The best conserved region in the sequence of these enzymes is located in their central section. This region contains a 30 conserved tryptophan residue which could be involved in the interaction with the glucan substrates [2] and it also contains a conserved glutamate which has been shown [3] to act as the nucleophile in the catalytic mechanism. this region was used as a signature pattern.

Consensus pattern: [LIVM]-x-[LIVMFYWA](3)-[STAG]-E-[STA]-G-W-P-[STN]-x-
[SAGO] [E is an active site residue]-

[1] Henrissat B. Biochem. J. 280:309-316(1991).

5 [2] Ori N., Sessa G., Lotan T., Himmelhoch S., Fluhr R. EMBO J. 9:3429-3436(1990).

[3] Varghese J.N., Garrett T.P.J., Colman P.M., Chen L., Hoj P.J., Fincher G.B. Proc. Natl.
Acad. Sci. U.S.A. 91:2785-2789(1994).

10 244. Glyoxalase I signatures

Glyoxalase I (EC 4.4.1.5) (lactoylglutathione lyase) catalyzes the first step of the glyoxal
pathway, the transformation of methylglyoxal and glutathione into S-lactoylglutathione which
is then converted by glyoxalase II to lactic acid [1]. Glyoxalase I is an ubiquitous enzyme
which binds one mole of zinc per subunit. The bacterial and yeast enzymes are monomeric
15 while the mammalian one is homodimeric. The sequence of glyoxalase I is well conserved. In
bacteria and mammals, the enzyme is a protein of about 130 to 180 residues while in fungi it
is about twice longer. In these organisms the enzyme is built out of the tandem repeat of an
homologous domain. Two signature patterns for this family were derived. The first one is
located in the N-terminal region while the second one is located in the central section of the
20 protein and contains a conserved histidine that could be implicated in the binding of the zinc
atom.

Consensus pattern: [HQ]-[IVT]-x-[LIVFY]-x-[IV]-x(5)-[STA]-x(2)-F-[YM]-x(2,3)-[LMF]-
G-[LMF]-

25 Consensus pattern: G-[NTKQ]-x(0,5)-[GA]-[LVFY]-[GH]-H-[IVF]-[CGA]-x-[STAGLE]-
x(2)-[DNC]-

[1] Kim N.-S., Umezawa Y., Ohmura S., Kato S. J. Biol. Chem. 268:11217-11221(1993).

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245. (Glypican)

Glypicans signature

Glypicans [1,2] are a family of heparan sulfate proteoglycans which are anchored to cell membranes by a glycosylphosphatidylinositol (GPI) linkage. Structurally, these proteins consist of three separate domains:

- a) A signal sequence;
- b) An extracellular domain of about 500 residues that contains 12 conserved cysteines probably involved in disulfide bonds and which also contains the sites of attachment of the heparan sulfate glycosaminoglycan side chains;
- c) A C-terminal hydrophobic region which is post-translationally removed after formation of the GPI-anchor.

The proteins known to belong to this family are:

- Glypican 1 (GPC1).
- Glypican 2 (GPC2) or cerebroglycan.
- Glypican 3 (GPC3) or OCL-5. In man, defects in GPC3 are the cause of a X-linked genetic disease, Simpson-Galabi-Behmel syndrome (SGBS).
- K-glypican.
- Glypican 5 (GPC5).
- Drosophila protein dally.

The signature pattern that was developed for glypicans is located in the central section of the extracellular domain and contains five of the conserved cysteines.

Consensus pattern C-x(2)-C-x-G-[LIVM]-x(4)-P-C-x(2)-[FY]-C-x(2)-[LIVM]-x(2)-G-C [The C's are probably involved in a disulfide bonds] Sequences known to belong to this class detected by the pattern ALL, except for dally.

[1] Weksberg R., Squire J.A., Templeton D.M. Nat. Genet. 12:225-227(1996).

[2] Watanabe K., Yamada H., Yamaguchi Y. J. Cell Biol. 130:1207-1218(1995).

Granins (chromogranins or secretogranins) [1] are a family of acidic proteins present in the secretory granules of a wide variety of endocrine and neuro-endocrine cells. The exact function(s) of these proteins is not yet known but they seem to be the precursors of biologically active peptides and/or they may act as helper proteins in the packaging of peptide hormones and neuropeptides. Three members of this family of proteins show some sequence similarities: - Chromogranin A (CGA) [2]. CGA is a protein of about 420 residues; it is the precursor of the peptide pancreastatin which strongly inhibits glucose- induced insulin release from the pancreas. - Secretogranin 1 (chromogranin B). A sulfated protein of about 600 residues. - Secretogranin 2 (chromogranin C). A sulfated protein of about 650 residues. Apart from their subcellular location and the abundance of acidic residues(Asp and Glu), these proteins do not share many structural similarities. Only one short region, located in the C-terminal section, is conserved in all these proteins. Chromogranins A and B share a region of high similarity in their N-terminal section; this region includes two cysteine residues involved in a disulfide bond

Consensus pattern: [DE]-[SN]-L-[SAN]-x(2)-[DE]-x-E-L-

Consensus pattern: C-[LIVM](2)-E-[LIVM](2)-S-[DN]-[STA]-L-x-K-x-S-x(3)- [LIVM]-[STA]-x-E-C [The two C's are linked by a disulfide bond]-

[1] Huttner W.B., Gerdes H.-H., Rosa P. Trends Biochem. Sci. 16:27-30(1991).

[2] Simon J.-P., Aunis D. Biochem. J. 262:1-13(1989).

247. grpE protein signature

In prokaryotes the grpE protein [1] stimulates, jointly with dnaJ, the ATPase activity of the dnaK chaperone. It seems to accelerate the release of ADP from dnaK thus allowing dnaK to recycle more efficiently. GrpE is a protein of about 22 to 25 Kd. In yeast, an evolutionary related mitochondrial protein(gene GRPE) has been shown [2] to associate with the mitochondrial hsp70protein and to thus play a role in the import of proteins from the cytoplasm. As a signature pattern, the most conserved region of grpE was selected. It is located in the C-terminal section.

Consensus pattern: [FL]-[DN]-[PHEA]-x(2)-[HM]-x-A-[LIVMTN]-x(16,20)-G-[FY]- x(3)-[DEG]-x(2)-[LIVM]-[RI]-x-[SA]-x-V-x-[IV]-

[1] Georgopoulos C., Welch W. *Annu. Rev. Cell Biol.* 9:601-635(1993).

[2] Bolliger L., Deloche O., Glick B.S., Georgopoulos C., Jenoe P., Kronidou N., Horst M., Morishima N., Schatz G. *EMBO J.* 13:1998-2006(1994).

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248. Guanylate kinase signature and profile

Guanylate kinase (EC 2.7.4.8) (GK) [1] catalyzes the ATP-dependent phosphorylation of GMP into GDP. It is essential for recycling GMP and indirectly, cGMP. In prokaryotes (such

- 10 as *Escherichia coli*), lower eukaryotes (such as yeast) and in vertebrates, GK is a highly conserved monomeric protein of about 200 amino acids. GK has been shown [2,3,4] to be structurally similar to the following proteins: - Protein A57R (or SalG2R) from various strains of *Vaccinia virus*. This protein is highly similar to GK, but contains a frameshift mutation in the N-terminal section and could therefore be inactive in that virus. The
- 15 following proteins are characterized by the presence in their sequence of one or more copies of the DHR domain, a SH3 domain (see <PDOC50002> as well as a C-terminal GK-like domain, these protein are collectively termed MAGUKs (membrane-associated guanylate kinase homologs) [5]: - *Drosophila lethal(1) discs large-1* tumor suppressor protein (gene *dlg1*). This protein is associated with septate junctions in developing flies and defects in the
- 20 *dlg1* gene cause neoplastic overgrowth of the imaginal disks. - Mammalian tight junction protein *Zo-1*. - A family of mammalian synaptic proteins that seem to interact with the cytoplasmic tail of NMDA receptor subunits. This family currently consist of *SAP90/PSD-95*, *CHAPSYN-110/PSD-93*, *SAP97/DLG1* and *SAP102*. - Vertebrate 55 Kd erythrocyte membrane protein (p55). p55 is a palmitoylated, membrane-associated protein of unknown
- 25 function. - *Caenorhabditis elegans* protein *lin-2*, which may play a structural role in the induction of the vulva. - Rat protein *CASK*. - Human protein *DLG2*. - Human protein *DLG3*. There is an ATP-binding site (P-loop) in the N-terminal section of GK. This region is not conserved in the GK-like domain of the above proteins which are therefore unlikely to be kinases. However these proteins retain the residues known, in GK, to be involved in the
- 30 binding of GMP. As a signature pattern a highly conserved region was selected that contains two arginine and a tyrosine which are involved in GMP-binding

Consensus pattern: T-[ST]-R-x(2)-[KR]-x(2)-[DE]-x(2)-G-x(2)-Y-x-[FY]-[LIVMK]-

- [1] Stehle T., Schulz G.E. J. Mol. Biol. 224:1127-1141(1992).
- [2] Bryant P.J., Woods D.F. Cell 68:621-622(1992).
- [3] Goebel M.G. Trends Biochem. Sci. 17:99-99(1992).
- [4] Zschocke P.D., Schiltz E., Schulz G.E. Eur. J. Biochem. 213:263-269(1993).
- 5 [5] Woods D.F., Bryant P.J. Mech. Dev. 44:85-89(1994).

249. (Glyco_hydro_35)

Glycosyl hydrolases family 35 putative active site

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Beta-galactosidases (EC 3.2.1.23) from mammals, fungi, plants and the bacteria *Xanthomonas manihotis* are evolutionary related [1,2]. They belong to family 35 in the classification of glycosyl hydrolases [3,E1].

15

Mammalian beta-galactosidase is a lysosomal enzyme (gene GLB1) which cleaves the terminal galactose from gangliosides, glycoproteins, and glycosaminoglycans and whose deficiency is the cause of the genetic disease Gm(1) gangliosidosis (Morquio disease type B).

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One of the best conserved regions in these enzymes contains a glutamic acid residue which, on the basis of similarities with other families of glycosyl hydrolases [4], probably acts as the proton donor in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern: G-G-P-[LIVM](2)-x(2)-Q-x-E-N-E-[FY] [The second E is the putative active site residue] Sequences known to belong to this class detected by the pattern ALL.

25

- [1] Taron C.H., Benner J.S., Hornstra L.J., Guthrie E.P. *Glycobiology* 5:603-610(1995).
- [2] Carey A.T., Holt K., Picard S., Wilde R., Tucker G.A., Bird C.R., Schuch W., Seymour G.B. *Plant Physiol.* 108:1099-1107(1995).
- [3] Henrissat B., Bairoch A. *Biochem. J.* 293:781-788(1993).
- 30 [4] Henrissat B., Callebaut I., Fabrega S., Lehn P., Mornon J.-P., Davies G. *Proc. Natl. Acad. Sci. U.S.A.* 92:7090-7094(1995).

250. (Glyco_hydro_16)

Glycosyl hydrolases family 16 signature

It has been shown [1] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities:

- Bacterial beta-1,3-1,4-glucanases, or lichenases, (EC 3.2.1.73) mainly from *Bacillus* but also from *Clostridium thermocellum* (gene *licB*), *Fibrobacter succinogenes* and *Rhodothermus marinus* (gene *bglA*).
- *Bacillus circulans* beta-1,3-glucanase A1 (EC 3.2.1.39) (gene *glcA*).
- Laminase (EC 3.2.1.6) from *Clostridium thermocellum* (gene *lam1*).
- *Streptomyces coelicolor* agarase (EC 3.2.1.81) (gene *dagA*).
- *Alteromonas carrageenovora* kappa-carrageenase (EC 3.2.1.83) (gene *cgkA*).

Two closely clustered conserved glutamates have been shown [2] to be involved in the catalytic activity of *Bacillus licheniformis* lichenase. The region that contains these residues as a signature pattern was used.

Consensus pattern E-[LIV]-D-[LIV]-x(0,1)-E-x(2)-[GQ]-[KRNF]-x-[PSTA] [The two E's are active site residues]

[1] Henrissat B. *Biochem. J.* 280:309-316(1991).

[2] Juncosa M., Pons J., Dot T., Querol E., Planas A. *J. Biol. Chem.* 269:14530-14535(1994).

251. (Glyco_hydro_17)

Glycosyl hydrolases family 17 signature

(aka glycosyl_hydro4)

It has been shown [1,2] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities:

- Glucan endo-1,3-beta-glucosidases (EC 3.2.1.39) (endo-(1->3)-beta-glucanase) from various plants. This enzyme may be involved in the defense of plants against pathogens through its ability to degrade fungal cell wall polysaccharides.

- Glucan 1,3-beta-glucosidase (EC 3.2.1.58) (exo-(1->3)-beta-glucanase) from yeast (gene BGL2). This enzyme may play a role in cell expansion during growth, in cell-cell fusion during mating, and in spore release during sporulation.

- Lichenases (EC 3.2.1.73) (endo-(1->3,1->4)-beta-glucanase) from various plants.

The best conserved region in the sequence of these enzymes is located in their central section.

This region contains a conserved tryptophan residue which could be involved in the interaction with the glucan substrates [2] and it also contains a conserved glutamate which has been shown [3] to act as the nucleophile in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern [LIVM]-x-[LIVMFYWA](3)-[STAG]-E-[STA]-G-W-P-[STN]-x-[SAGO]
[E is an active site residue] Sequences known to belong to this class detected by the pattern ALL.

[1] Henrissat B. Biochem. J. 280:309-316(1991).

[2] Ori N., Sessa G., Lotan T., Himmelhoch S., Fluhr R. EMBO J. 9:3429-3436(1990).

[3] Varghese J.N., Garrett T.P.J., Colman P.M., Chen L., Hoj P.J., Fincher G.B. Proc. Natl. Acad. Sci. U.S.A. 91:2785-2789(1994).

252. (Glyco_hydro_3)

Glycosyl hydrolases family 3 active site

It has been shown [1,2] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

- Beta glucosidases (EC 3.2.1.21) from the fungi *Aspergillus wentii* (A-3), *Hansenula anomala*, *Kluyveromyces fragilis*, *Saccharomycopsis fibuligera*, (BGL1 and BGL2), *Schizophyllum commune* and *Trichoderma reesei* (BGL1).
- Beta glucosidases from the bacteria *Agrobacterium tumefaciens* (Cbg1),

Butyrivibrio fibrisolvens (bglA), Clostridium thermocellum (bglB), Escherichia coli (bglX), Erwinia chrysanthemi (bgxA) and Ruminococcus albus.

- Alteromonas strain O-7 beta-hexosaminidase A (EC 3.2.1.52).
- Bacillus subtilis hypothetical protein yzbA.
- Escherichia coli hypothetical protein ycfO and HI0959, the corresponding Haemophilus influenzae protein.

One of the conserved regions in these enzymes is centered on a conserved aspartic acid residue which has been shown [3], in Aspergillus wentii beta- glucosidase A3, to be implicated in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern[LIVM](2)-[KR]-x-[EQK]-x(4)-G-[LIVMFT]-[LIVT]-[LIVMF]-[ST]-D-x(2)-[SGADNI] [D is the active site residue] Sequences known to belong to this class detected by the patternALL.

- [1] Henrissat B. Biochem. J. 280:309-316(1991).
- [2] Castle L.A., Smith K.D., Morris R.O. J. Bacteriol. 174:1478-1486(1992).
- [3] Bause E., Legler G. Biochim. Biophys. Acta 626:459-465(1980).

253. (Glyco_hydro_28)

Polygalacturonase active site (aka PG)

Polygalacturonase (EC 3.2.1.15) (PG) (pectinase) [1,2] catalyzes the random hydrolysis of 1,4-alpha-D-galactosiduronic linkages in pectate and other galacturonans. In fruit, polygalacturonase plays an important role in cell wall metabolism during ripening. In plant bacterial pathogens such as Erwinia carotovora or Pseudomonas solanacearum and fungal pathogens such as Aspergillus niger, polygalacturonase is involved in maceration and soft-rotting of plant tissue.

Exo-poly-alpha-D-galacturonosidase (EC 3.2.1.82) (exoPG) [3] hydrolyzes peptic acid from the non-reducing end, releasing digalacturonate.

Prokaryotic, eukaryotic PG and exoPG share a few regions of sequence similarity. The best conserved of these regions was selected. It is centered on a conserved histidine most probably involved in the catalytic mechanism [4].

- 5 Consensus pattern[GSDENKRH]-x(2)-[VMFC]-x(2)-[GS]-H-G-[LIVMAG]-x(1,2)-[LIVM]-G-S [H is the putative active site residue] Sequences known to belong to this class detected by the patternALL.

Note: these proteins belong to family 28 in the classification of glycosyl hydrolases [5].

10

[1] Ruttowski E., Labitzke R., Khanh N.Q., Loeffler F., Gottschalk M., Jany K.-D. Biochim. Biophys. Acta 1087:104-106(1990).

[2] Huang J., Schell M.A. J. Bacteriol. 172:3879-3887(1990).

[3] He S.Y., Collmer A. J. Bacteriol. 172:4988-4995(1990).

- 15 [4] Bussink H.J.D., Buxton F.P., Visser J. Curr. Genet. 19:467-474(1991).

[5] Henrissat B. Biochem. J. 280:309-316(1991).

254. (Glyco_hydro_32)

- 20 Glycosyl hydrolases family 32 active site

It has been shown [1,2] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities:

- 25 - Inulinase (EC 3.2.1.7) (or inulase) from the fungi *Kluyveromyces marxianus*.
 - Beta-fructofuranosidase (EC 3.2.1.26), commonly known as invertase in fungi and plants and as sucrase in bacteria (gene *sacA* or *scrB*).
 - Raffinose invertase (EC 3.2.1.26) (gene *rafD*) from *Escherichia coli* plasmid pRSD2.
 30 - Levanase (EC 3.2.1.65) (gene *sacC*) from *Bacillus subtilis*.

One of the conserved regions in these enzymes is located in the N-terminal section and contains an aspartic acid residue which has been shown [3], in yeast invertase to be important for the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern H-x(2)-P-x(4)-[LIVM]-N-D-P-N-G [D is the active site residue]

Sequences known to belong to this class detected by the pattern ALL.

- 5 [1] Henrissat B. Biochem. J. 280:309-316(1991).
- [2] Gunasekaran P., Karunakaran T., Cami B., Mukundan A.G., Preziosi L., Baratti J. J. Bacteriol. 172:6727-6735(1990).
- [3] Reddy V.A., Maley F. J. Biol. Chem. 265:10817-10120(1990).

10

255. (Glyco_hydro_1)

Glycosyl hydrolases family 1 signatures

15

It has been shown [1 to 4] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

20

- Beta-glucosidases (EC 3.2.1.21) from various bacteria such as *Agrobacterium* strain ATCC 21400, *Bacillus polymyxa*, and *Caldocellum saccharolyticum*.

- Two plants (clover) beta-glucosidases (EC 3.2.1.21).

25

- Two different beta-galactosidases (EC 3.2.1.23) from the archaeobacteria *Sulfolobus solfataricus* (genes *bgaS* and *lacS*).

- 6-phospho-beta-galactosidases (EC 3.2.1.85) from various bacteria such as *Lactobacillus casei*, *Lactococcus lactis*, and *Staphylococcus aureus*.

- 6-phospho-beta-glucosidases (EC 3.2.1.86) from *Escherichia coli* (genes *bgIB* and *ascB*) and from *Erwinia chrysanthemi* (gene *arbB*).

- Plants myrosinases (EC 3.2.3.1) (sinigrinase) (thioglucosidase).

- Mammalian lactase-phlorizin hydrolase (LPH) (EC 3.2.1.108 / EC 3.2.1.62).

30

LPH, an integral membrane glycoprotein, is the enzyme that splits lactose in the small intestine. LPH is a large protein of about 1900 residues which contains four tandem repeats of a domain of about 450 residues which is evolutionary related to the above glycosyl hydrolases.

One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [5], in the beta-glucosidase from *Agrobacterium*, to be directly

involved in glycosidic bond cleavage by acting as a nucleophile. This region was used as a signature pattern. As a second signature pattern we selected a conserved region, found in the N-terminal extremity of these enzymes, this region also contains a glutamic acid residue.

- 5 Consensus pattern[LIVMFSTC]-[LIVFYS]-[LIV]-[LIVMST]-E-N-G-[LIVMFAR]-[CSAGN] [E is the active site residue] Sequences known to belong to this class detected by the patternALL.

- Note: this pattern will pick up the last two domains of LPH; the first two domains, which are removed from the LPH precursor by proteolytic processing, have lost the active site glutamate and may therefore be inactive [4].
- 10

Consensus patternF-x-[FYWM]-[GSTA]-x-[GSTA]-x-[GSTA](2)-[FYNH]-[NQ]-x-E-x-[GSTA] Sequences known to belong to this class detected by the pattern ALL.

- 15 Note: this pattern will pick up the last three domains of LPH.

- [1] Henrissat B. Biochem. J. 280:309-316(1991).
 [2] Henrissat B. Protein Seq. Data Anal. 4:61-62(1991).
 20 [3] Gonzalez-Candelas L., Ramon D., Polaina J. Gene 95:31-38(1990).
 [4] El Hassouni M., Henrissat B., Chippaux M., Barras F. J. Bacteriol. 174:765-777(1992).
 [5] Withers S.G., Warren R.A.J., Street I.P., Rupitz K., Kempton J.B., Aebersold R. J. Am. Chem. Soc. 112:5887-5889(1990).

25

256. Glyco_hydro_20

Glycosyl hydrolase family 20

Previous Pfam IDs: glycosyl_hydr11;

Number of members: 33

30

257. (Glyco_hydro_9)

Glycosyl hydrolases family 9 active sites signatures

(aka Glycosyl_hydr12)

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1,2]. Fungi and bacteria produces a spectrum of cellulolytic enzymes (cellulases) and xylanases which, on the basis of sequence similarities, can be classified into families. One of these families is known as the cellulase family E [3] or as the glycosyl hydrolases family 9 [4,E1]. The enzymes which are currently known to belong to this family are listed below.

- 10 - *Butyrivibrio fibrisolvens* cellodextrinase 1 (ced1).
- *Cellulomonas fimi* endoglucanases B (cenB) and C (cenC).
- *Clostridium cellulolyticum* endoglucanase G (celCCG).
- *Clostridium cellulovorans* endoglucanase C (engC).
- *Clostridium stercoarium* endoglucanase Z (avicelase I) (celZ).
- 15 - *Clostridium thermocellum* endoglucanases D (celD), F (celF) and I (celI).
- *Fibrobacter succinogenes* endoglucanase A (endA).
- *Pseudomonas fluorescens* endoglucanase A (celA).
- *Streptomyces reticuli* endoglucanase 1 (cel1).
- *Thermomonospora fusca* endoglucanase E-4 (celD).
- 20 - *Dictyostelium discoideum* spore germination specific endoglucanase 270-6. This slime mold enzyme may digest the spore cell wall during germination, to release the enclosed amoeba.
- Endoglucanases from plants such as Avocado or French bean. In plants this enzyme may be
- 25 involved in the fruit ripening process.

Two of the most conserved regions in these enzymes are centered on conserved residues which have been shown [5,6], in the endoglucanase D from *Cellulomonas thermocellum*, to be important for the catalytic activity. The first region contains an active site histidine and the

30 second region contains two catalytically important residues: an aspartate and a glutamate. Both regions were used as signature patterns.

Consensus pattern [STV]-x-[LIVMFY]-[STV]-x(2)-G-x-[NKR]-x(4)-[PLIVM]-H-x-R [H is an active site residue] Sequences known to belong to this class detected by the pattern ALL, except for *Cellulomonas fimi* cenC and *Streptomyces reticuli* cell.

- 5 Consensus pattern [FYW]-x-D-x(4)-[FYW]-x(3)-E-x-[STA]-x(3)-N-[STA] [D and E are active site residues] Sequences known to belong to this class detected by the pattern ALL, except for *Fibrobacter succinogenes* endA whose sequence seems to be incorrect.

[1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).

- 10 [2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).

[3] Henrissat B., Claeysens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).

[4] Henrissat B. Biochem. J. 280:309-316(1991).

- [5] Tomme P., Chauvaux S., Beguin P., Millet J., Aubert J.-P., Claeysens M. J. Biol. Chem. 15 266:10313-10318(1991).

[6] Tomme P., van Beeumen J., Claeysens M. Biochem. J. 285:319-324(1992).

258. Matrix protein (MA), p15 (GAG_ma)

- 20 The matrix protein, p15, is encoded by the gag gene. MA is involved in pathogenicity [1].

[1] : Pozsgay JM, Beilharz MW, Wines BD, Hess AD, Pitha PM, J Virol 1993;67:5989-5999.

25

259. Gag polyprotein, inner coat protein p12 (GAG_P12)

The retroviral p12 is a virion structural protein. p12 is proline rich. The function carried out by p12 in assembly and replication is unknown. p12C is associated with pathogenicity of the virus

- 30 [1] Pozsgay JM, Beilharz MW, Wines BD, Hess AD, Pitha PM, J Virol 1993;67:5989-5999.

260. Glutamine synthetase signatures (GLN-SYNT)

Glutamine synthetase (EC 6.3.1.2) (GS) [1] plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine. There seem to be three different classes of GS [2,3,4]: - Class I enzymes (GSI) are specific to prokaryotes, and are oligomers of 12 identical subunits. The activity of GSI-type enzyme is controlled by the adenylation of a tyrosine residue. The adenylation of the enzyme is inactive. - Class II enzymes (GSII) are found in eukaryotes and in bacteria belonging to the Rhizobiaceae, Frankiaceae, and Streptomycetaceae families (these bacteria have also a class-I GS). GSII are octamer of identical subunits. Plants have two or more isozymes of GSII, one of the isozymes is translocated into the chloroplast. - Class III enzymes (GSIII) has, currently, only been found in *Bacteroides fragilis* and in *butyrivibrio fibrisolvens*. It is a hexamer of identical chains. It is much larger (about 700 amino acids) than the GSI (450 to 470 amino acids) or GSII (350 to 420 amino acids) enzymes. While the three classes of GS's are clearly structurally related, the sequence similarities are not so extensive. As signature patterns three conserved regions were selected. The first pattern is based on a conserved tetrapeptide in the N-terminal section of the enzyme, the second one is based on a glycine-rich region which is thought to be involved in ATP-binding. The third pattern is specific to class I glutamine synthetases and includes the tyrosine residue which is reversibly adenylation.

Consensus pattern: [FYWL]-D-G-S-S-x(6,8)-[DENQSTAK]-[SA]-[DE]-x(2)-[LIVMFY]-
 Consensus pattern: K-P-[LIVMFYA]-x(3,5)-[NPAT]-G-[GSTAN]-G-x-H-x(3)-S-
 Consensus pattern: K-[LIVM]-x(5)-[LIVMA]-D-[RK]-[DN]-[LI]-Y [Y is the site of
 adenylation]-

- [1] Eisenberg D., Almassy R.J., Janson C.A., Chapman M.S., Suh S.W., Cascio D., Smith W.W. Cold Spring Harbor Symp. Quant. Biol. 52:483-490(1987).
 [2] Kumada Y., Benson D.R., Hillemann D., Hosted T.J., Rochefort D.A., Thompson C.J., Wohlleben W., Tateno Y. Proc. Natl. Acad. Sci. U.S.A. 90:3009-3013(1993).
 [3] Shatters R.G., Kahn M.L. J. Mol. Evol. 29:422-428(1989).
 [4] Brown J.R., Masuchi Y., Robb F.T., Doolittle W.F. J. Mol. Evol. 38:566-576(1994).

Globins are heme-containing proteins involved in binding and/or transporting oxygen [1].

They belong to a very large and well studied family which is widely distributed in many organisms. The major groups of globins are: - Hemoglobins (Hb) from vertebrates. Hb is the protein responsible for transporting oxygen from the lungs to other tissues. It is a tetramer of

- 5 two alpha and two beta chains. Most vertebrate species also express specific embryonic or fetal forms of hemoglobin where the alpha or the beta chains are replaced by a chain with higher oxygen affinity, as for the gamma, delta, epsilon and zeta chains in mammals, for example. - Myoglobins (Mg) from vertebrates. Mg is a monomeric protein responsible for oxygen storage in muscles. - Invertebrate globins [2]. A wide variety of globins are found in
- 10 invertebrates. Molluscs generally have one or two muscle globins which are either monomeric or dimeric. Insects, such as the midge *Chironomus thummi*, have a large set of extracellular globins. Nematodes and annelids have a variety of intracellular and extracellular globins; some of them are multi- domain polypeptides (from two up to nine-domain globins) and some produce large, disulfide-bonded aggregates. - Leghemoglobins (Lg) from the root
- 15 nodules of leguminous plants. Lg provides oxygen for bacteroids. - Flavohemoproteins from bacteria (*Escherichia coli hmpA*) and fungi [3]. These proteins consist of two distinct domains: an N-terminal globin domain and a C-terminal FAD-containing reductase domain. In bacteria such as *Vitreoscilla*, the enzyme-associated globin is a single domain protein. All these globins seem to have evolved from a common ancestor. The profile developed to detect
- 20 members of the globin family is based on a structural alignment of selected globin sequences [1] Concise Encyclopedia Biochemistry, Second Edition, Walter de Gruyter, Berlin New-York (1988).[2] Goodman M., Pedwaydon J., Czelusniak J., Suzuki T., Gotoh T., Moens L., Shishikura F., Walz D., Vinogradov S. J. Mol. Evol. 27:236-249(1988).

25 Plant hemoglobins signature (globin2)

Leghemoglobins [1] are hemoproteins present in the root nodules of leguminous plants.

Leghemoglobins are structurally and functionally related to hemoglobin and myoglobin. By providing oxygen to the bacteroids, they are essential for symbiotic nitrogen fixation.

- 30 Structurally related hemoglobins from the nodules of non-leguminous plants [2,3], and from the roots of non-nodulating plants[4] have been recently sequenced. A signature pattern was developed that picks up the sequence of plants hemoglobins, exclusively.

Consensus pattern: [SN]-P-x-L-x(2)-H-A-x(3)-F-

- [1] Powell R., Gannon F. *BioEssays* 9:117-121(1988).
 [2] Kortt A.A., Trinick M.J., Appleby C.A. *Eur. J. Biochem.* 175:141-149(1988).
 [3] Kortt A.A., Inglis A.S., Fleming A.I., Appleby C.A. *FEBS Lett.* 231:341-346(1988).
 [4] Bogusz D., Appleby C.A., Landsmann J., Dennis E.S., Trinick M.J., Peacock W.J.
 5 *Nature* 331:178-180(1988).

262. Fructose-bisphosphate aldolase class-I active site (glycolytic_enz)

Fructose-bisphosphate aldolase [1,2] is a glycolytic enzyme that catalyzes the
 10 reversible aldol cleavage or condensation of fructose-1,6-bisphosphate into
 dihydroxyacetone-phosphate and glyceraldehyde 3-phosphate. There are two classes of
 fructose-bisphosphate aldolases with different catalytic mechanisms. Class-I aldolases [3],
 mainly found in higher eukaryotes, are homotetrameric enzymes which form a Schiff-base
 intermediate between the C-2 carbonyl group of the substrate (dihydroxyacetone
 15 phosphate) and the epsilon-amino group of a lysine residue. In vertebrates, three forms of this
 enzyme are found: aldolase A in muscle, aldolase B in liver and aldolase C in brain. The
 sequence around the lysine involved in the Schiff-base is highly conserved and can be used as
 a signature for this class of enzyme.

20 Consensus pattern: [LIVM]-x-[LIVMFYW]-E-G-x-[LS]-L-K-P-[SN] [K is involved in
 Schiff-base formation]-

- [1] Perham R.N. *Biochem. Soc. Trans.* 18:185-187(1990).
 [2] Marsh J.J., Lebherz H.G. *Trends Biochem. Sci.* 17:110-113(1992).
 25 [3] Freemont P.S., Dunbar B., Fothergill-Gilmore L.A. *Biochem. J.* 249:779-788(1988).

263. Glycosyl hydrolases family 11 active sites signatures

The microbial degradation of cellulose and xylans requires several types of enzymes such as
 30 endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases
 (EC 3.2.1.8) [1,2]. Fungi and bacteria produces a spectrum of cellulolytic enzymes
 (cellulases) and xylanases which, on the basis of sequence similarities, can be classified into
 families. One of these families is known as the cellulase family G [3] or as the glycosyl
 hydrolases family 11 [4,E1]. The enzymes which are currently known to belong to this family

are listed below. - *Aspergillus awamori* xylanase C (xynC). - *Bacillus circulans*, *pumilus*, *stearothermophilus* and *subtilis* xylanase (xynA). - *Clostridium acetobutylicum* xylanase (xynB). - *Clostridium stercorarium* xylanase A (xynA). - *Fibrobacter succinogenes* xylanase C (xynC) which consist of two catalytic domains that both belong to family 10. -

- 5 Neocallimastix patriciarum xylanase A (xynA). - *Ruminococcus flavefaciens* bifunctional xylanase XYLA (xynA). This protein consists of three domains: a N-terminal xylanase catalytic domain that belongs to family 11 of glycosyl hydrolases; a central domain composed of short repeats of Gln, Asn and Trp, and a C-terminal xylanase catalytic domain that belongs to family 10 of glycosyl hydrolases. - *Schizophyllum commune* xylanase A. -
- 10 *Streptomyces lividans* xylanases B (xlnB) and C (xlnC). - *Trichoderma reesei* xylanases I and II. Two of the conserved regions in these enzymes are centered on glutamic acid residues which have both been shown [5], in *Bacillus pumilis* xylanase, to be necessary for catalytic activity. Both regions were used as signature patterns.

- 15 Consensus pattern: [PSA]-[LQ]-x-E-Y-Y-[LIVM](2)-[DE]-x-[FYWHN] [E is an active site residue]-
 Consensus pattern: [LIVMF]-x(2)-E-[AG]-[YWG]-[QRFGS]-[SG]-[STAN]-G-x-[SAF] [E is an active site residue]-

- 20 [1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).
 [2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).
 [3] Henrissat B., Claeysens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).
 [4] Henrissat B. Biochem. J. 280:309-316(1991).
 25 [5] Ko E.P., Akatsuka H., Moriyama H., Shinmyo A., Hata Y., Katsube Y., Urabe I., Okada H. Biochem. J. 288:117-121(1992).

264. Glycosyl hydrolase family 14

- 30 This family are beta amylases.

265. Glycosyl hydrolases family 1 signatures

It has been shown [1 to 4] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family: - Beta-glucosidases (EC 3.2.1.21) from various bacteria such as *Agrobacterium* strain ATCC 21400, *Bacillus polymyxa*, and *Caldocellum saccharolyticum*. - Two plants (clover) beta-glucosidases (EC 3.2.1.21). - Two different beta-galactosidases (EC 3.2.1.23) from the archaeobacteria *Sulfolobus solfataricus* (genes *bgaS* and *lacS*). - 6-phospho-beta-galactosidases (EC 3.2.1.85) from various bacteria such as *Lactobacillus casei*, *Lactococcus lactis*, and *Staphylococcus aureus*. - 6-phospho-beta-glucosidases (EC 3.2.1.86) from *Escherichia coli* (genes *bgIB* and *ascB*) and from *Erwinia chrysanthemi* (gene *arbB*). - Plants myrosinases (EC 3.2.3.1) (sinigrinase) (thioglucosidase). - Mammalian lactase-phlorizin hydrolase (LPH) (EC 3.2.1.108 / EC 3.2.1.62). LPH, an integral membrane glycoprotein, is the enzyme that splits lactose in the small intestine. LPH is a large protein of about 1900 residues which contains four tandem repeats of a domain of about 450 residues which is evolutionary related to the above glycosyl hydrolases. One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [5], in the beta-glucosidase from *Agrobacterium*, to be directly involved in glycosidic bond cleavage by acting as a nucleophile. This region was used as a signature pattern. As a second signature pattern a conserved region was selected, found in the N-terminal extremity of these enzymes, this region also contains a glutamic acid residue.

Consensus pattern: [LIVMFSTC]-[LIVFYS]-[LIV]-[LIVMST]-E-N-G-[LIVMFAR]-[CSAGN] [E is the active site residue]

Note: this pattern will pick up the last two domains of LPH; the first two domains, which are removed from the LPH precursor by proteolytic processing, have lost the active site glutamate and may therefore be inactive [4].

Consensus pattern: F-x-[FYWM]-[GSTA]-x-[GSTA]-x-[GSTA](2)-[FYNH]-[NQ]-x-E-x-[GSTA]-

[1] Henrissat B. *Biochem. J.* 280:309-316(1991).

[2] Henrissat B. *Protein Seq. Data Anal.* 4:61-62(1991).

[3] Gonzalez-Candelas L., Ramon D., Polaina J. *Gene* 95:31-38(1990).

[4] El Hassouni M., Henrissat B., Chippaux M., Barras F. J. *Bacteriol.* 174:765-777(1992).

[5] Withers S.G., Warren R.A.J., Street I.P., Rupitz K., Kempton J.B., Aebersold R. J. *Am. Chem. Soc.* 112:5887-5889(1990).

266. Glycosyl hydrolases family 2 signatures

It has been shown [1,2,E1] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family: - Beta-galactosidases (EC 3.2.1.23) from bacteria such as *Escherichia coli* (genes *lacZ* and *ebgA*), *Clostridium acetobutylicum*, *Clostridium thermosulfurogenes*, *Klebsiella pneumoniae*, *Lactobacillus delbrueckii*, or *Streptococcus thermophilus* and from the fungi *Kluyveromyces lactis*. - Beta-glucuronidase (EC 3.2.1.31) from *Escherichia coli* (gene *uidA*) and from mammals. One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [3], in *Escherichia coli lacZ*, to be the general acid/base catalyst in the active site of the enzyme. This region was used as a signature pattern. As a second signature pattern a highly conserved region was selected located some sixty residues upstream from the active site glutamate.

Consensus pattern: N-x-[LIVMFYWD]-R-[STACN](2)-H-Y-P-x(4)-[LIVMFYWS](2)-x(3)-[DN]-x(2)-G-[LIVMFYW](4)-

Consensus pattern: [DENOLF]-[KRVW]-N-[HRY]-[STAPV]-[SAC]-[LIVMFS](3)-W-[GS]-x(2,3)-N-E [E is the active site residue]-

[1] Henrissat B. *Biochem. J.* 280:309-316(1991).

[2] Schroeder C.J., Robert C., Lenzen G., McKay L.L., Mercenier A. J. *Gen. Microbiol.* 137:369-380(1991).

[3] Gebler J.C., Aebersold R., Withers S.G. *J. Biol. Chem.* 267:11126-11130(1992).

267. Glycosyl hydrolases family 3 active site

It has been shown [1,2] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

- Beta glucosidases (EC 3.2.1.21) from the fungi *Aspergillus wentii* (A-3), *Hansenula anomala*, *Kluyveromyces fragilis*, *Saccharomycopsis fibuligera*, (BGL1 and BGL2), *Schizophyllum commune* and *Trichoderma reesei* (BGL1).
- Beta glucosidases from the bacteria *Agrobacterium tumefaciens* (Cbg1), *Butyrivibrio fibrisolvens* (bglA), *Clostridium thermocellum* (bglB),

Escherichia coli (bglX), *Erwinia chrysanthemi* (bgxA) and *Ruminococcus albus*. - *Alteromonas* strain O-7 beta-hexosaminidase A (EC 3.2.1.52).

- *Bacillus subtilis* hypothetical protein yzbA.

- *Escherichia coli* hypothetical protein ycfO and HI0959, the corresponding

5 *Haemophilus influenzae* protein.

One of the conserved regions in these enzymes is centered on a conserved aspartic acid residue which has been shown [3], in *Aspergillus wentii* beta-glucosidase A3, to be implicated in the catalytic mechanism. This region was used as a signature pattern.

10

Consensus pattern: [LIVM](2)-[KR]-x-[EOK]-x(4)-G-[LIVMFT]-[LIVT]-[LIVMF]-[ST]-D-x(2)-[SGADNI] [D is the active site residue]

[1] Henrissat B. *Biochem. J.* 280:309-316(1991).

15 [2] Castle L.A., Smith K.D., Morris R.O. *J. Bacteriol.* 174:1478-1486(1992).

[3] Bause E., Legler G. *Biochim. Biophys. Acta* 626:459-465(1980).

268. Glycosyl hydrolases family 8 signature

20 The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91)(exoglucanases), or xylanases (EC 3.2.1.8) [1,2]. Fungi and bacteria produces a spectrum of cellulolytic enzymes (cellulases) and xylanases which, on the basis of sequence similarities, can be classified into families. One of these families is known as the cellulase family D [3] or as the glycosyl

25 hydrolases family 8 [4,E1]. The enzymes which are currently known to belong to this family are listed below. - *Acetobacter xylinum* endonuclease cmcAX. - *Bacillus* strain KSM-330 acidic endonuclease K (Endo-K). - *Cellulomonas josui* endoglucanase 2 (celB). - *Cellulomonas uda* endoglucanase. - *Clostridium cellulolyticum* endoglucanases C (celcCC). - *Clostridium thermocellum* endoglucanases A (celA). - *Erwinia chrysanthemi* minor

30 endoglucanase γ (celY). - *Bacillus circulans* beta-glucanase (EC 3.2.1.73). - *Escherichia coli* hypothetical protein yhjM. The most conserved region in these enzymes is a stretch of about 20 residues that contains two conserved aspartate. The first aspartate is thought [5] to act as the nucleophile in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern: A-[ST]-D-x(2)-[IM]-A-x-[SA]-[LIVM]-[LIVMG]-x-A-x(3)-
[FW] [The first D is an active site residue]-

[1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).

5 [2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).

[3] Henrissat B., Claeyssens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).

[4] Henrissat B. Biochem. J. 280:309-316(1991).

[5] Alzari P.M., Souchon H., Dominguez R. Structure 4:265-275(1996).

10

269. Glycosyl hydrolases family 9 active sites signatures

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases

15 (EC 3.2.1.8) [1,2]. Fungi and bacteria produce a spectrum of cellulolytic enzymes (cellulases)

and xylanases which, on the basis of sequence similarities, can be classified into families.

One of these families is known as the cellulase family E [3] or as the glycosyl hydrolases family 9 [4, E1]. The enzymes which are currently known to belong to this family are listed

below. - *Butyrivibrio fibrisolvens* cellobiohydrolase 1 (ced1). - *Cellulomonas fimi*

20 endoglucanases B (cenB) and C (cenC). - *Clostridium cellulolyticum* endoglucanase G (celCCG). - *Clostridium cellulovorans* endoglucanase C (engC). - *Clostridium stercoarum* endoglucanase Z (avicelase I) (celZ). - *Clostridium thermocellum* endoglucanases D (celD), F (celF) and I (celI). - *Fibrobacter succinogenes* endoglucanase A (endA). - *Pseudomonas fluorescens* endoglucanase A (celA). - *Streptomyces reticuli* endoglucanase 1 (cel1). -

25 *Thermomonospora fusca* endoglucanase E-4 (celD). - *Dictyostelium discoideum* spore germination specific endoglucanase 270-6. This slime mold enzyme may digest the spore cell wall during germination, to release the enclosed amoeba. - Endoglucanases from plants such as Avocado or French bean. In plants this enzyme may be involved the fruit ripening process. Two of the most conserved regions in these enzymes are centered on conserved residues

30 which have been shown [5,6], in the endoglucanase D from *Cellulomonas thermocellum*, to be important for the catalytic activity. The first region contains an active site histidine and the second region contains two catalytically important residues: an aspartate and a glutamate.

Both regions were used as signature patterns.

Consensus pattern: [STV]-x-[LIVMFY]-[STV]-x(2)-G-x-[NKR]-x(4)-[PLIVM]-H-x-R [H is an active site residue]-

Consensus pattern: [FYW]-x-D-x(4)-[FYW]-x(3)-E-x-[STA]-x(3)-N-[STA] [D and E are active site residues]-

5

[1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).

[2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).

[3] Henrissat B., Claeysens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).

10 [4] Henrissat B. Biochem. J. 280:309-316(1991).

[5] Tomme P., Chauvaux S., Beguin P., Millet J., Aubert J.-P., Claeysens M. J. Biol. Chem. 266:10313-10318(1991).

[6] Tomme P., van Beeumen J., Claeysens M. Biochem. J. 285:319-324(1992).

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270. Glyceraldehyde 3-phosphate dehydrogenase active site (gpdh)

Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) (GAPDH) [1] is a tetrameric

NAD-binding enzyme common to both the glycolytic and gluconeogenic pathways. A

cysteine in the middle of the molecule is involved in forming a covalent phosphoglycerol

20 thioester intermediate. The sequence around this cysteine is totally conserved in eubacterial

and eukaryotic GAPDHs and is also present, albeit in a variant form, in the otherwise highly

divergent archaeobacterial GAPDH [2]. Escherichia coli D-erythrose 4-phosphate

dehydrogenase (E4PDH) (gene epd orgapB) is an enzyme highly related to GAPDH [3].

25 Consensus pattern: [ASV]-S-C-[NT]-T-x(2)-[LIM] [C is the active site residue]-

[1] Harris J.I., Waters M. (In) The Enzymes (3rd edition) 13:1-50(1976).

[2] Fabry S., Lang J., Niermann T., Vingron M., Hensel R. Eur. J. Biochem. 179:405-413(1989).

30 [3] Zhao G., Pease A.J., Bharani N., Winkler M.E. J. Bacteriol. 177:2804-2812(1995).

271. Granulins signature

Granulins [1] are a family of cysteine-rich peptides of about 6 Kd which may have multiple biological activity. A precursor protein (known as acrogranin) potentially encodes seven different forms of granulin (grnA to grnG) which are probably released by post-translational proteolytic processing. A schematic representation of the structure of a granulin is shown below: xxxCxxxxxCxxxxCCxxxxxxxxCCxxxxxCxxxxxCxxxxxCxxxxxCx
 *****'C': conserved cysteine probably involved in a disulfide bond.'*': position of the pattern. Granulins are evolutionary related to a PMP-D1, a peptide extracted from the pars intercerebralis of migratory locusts [2].

Consensus pattern: C-x-D-x(2)-H-C-C-P-x(4)-C [The four C's are probably involved in disulfide bonds]-

[1] Bhandari V., Palfree R.G., Bateman A. Proc. Natl. Acad. Sci. U.S.A. 89:1715-1719(1992).

[2] Nakakura N., Hietter H., van Dorsselaer A., Luu B. Eur. J. Biochem. 204:147-153(1992).

272. (HCV RdRp) Hepatitis C virus RNA dependent RNA polymerase

The RNA dependent RNA polymerase is also known as non-structural protein NS5B. NS5B is a 65 kDa protein that resembles other viral RNA polymerases. HCV replication is thought to occur in membrane bound replication complexes. These complexes transcribe the positive strand and the resulting minus strand is used as a template for the synthesis of genomic RNA. There are two viral proteins involved in the reaction, NS3 and NS5B.[1,2]

[1] Lohmann V, Korner F, Herian U, Bartenschlager R;

J Virol 1997;71:8416-8428. [2] Behrens SE, Tomei L, De Francesco R; EMBO J 1996;15:12-22. [3] Ishido S, Fujita T, Hotta H; Biochem Biophys Res Commun 1998;244:35-40.

273. (HHH) Helix-hairpin-helix motif.

[1] Doherty AJ, Serpell LC, Ponting CP; Nucleic Acids Res 1996;24:2488-2497.

5

274. HIT family signature

Recently a family of small proteins of about 12 to 16 Kd has been described[1]. This family currently consists of: - Mammalian protein HINT (also known as Protein kinase C inhibitor 1 or PKCI- 1). HINT was incorrectly thought to be a specific inhibitor of PKC. It has been
 10 shown to bind zinc. - Fission yeast diadenosine 5',5'''-P₁,P₄-tetraphosphate asymmetrical hydrolase (Ap4Aase) (EC 3.6.1.17) [2] (gene aph1), which cleaves A-5'-PPPP- 5'A to yield AMP and ATP. - FHIT, a human protein whose gene is altered in different tumors and which acts [3] as a diadenosine 5',5'''-P₁,P₃-triphosphate hydrolase (Ap3Aase) (EC 3.6.1.29)
 15 cleaving A-5'-PPP-5'A to yield AMP and ADP. - Yeast proteins HNT1 and HNT2. - Maize zinc-binding protein ZBP14. - Escherichia coli hypothetical protein ycfF. - Haemophilus influenzae hypothetical protein HI0961. - Helicobacter pylori hypothetical protein HP0404. - Methanococcus jannaschii hypothetical protein MJ0866. - Mycobacterium leprae hypothetical protein U296A. - Synechocystis strain PCC 6803 hypothetical protein slr1234. - Caenorhabditis elegans hypothetical protein F21C3.3. - A hypothetical 13.2 Kd protein in
 20 hisE 3'region in Azospirillum brasilense. - A hypothetical 13.1 Kd protein in p37 5'region in Mycoplasma hyorhinis. - A hypothetical 12.4 Kd protein in psbAII 5'region in Synechococcus strain PCC 7942. All these proteins contains a region with three clustered histidines. This region is responsible for the designation of this family: HIT, for 'HlsthidineTriad' [1]. This region was originally thought to be implied in the binding of a zinc
 25 ion but was later identified [4] as part of the alpha-phosphate binding site of a nucleotide-binding domain. As a signature pattern, the region of the histidine triad was selected.

Consensus pattern: [NOA]-x(4)-[GAV]-x-[QF]-x-[LIVM]-x-H-[LIVMFYT]-H-[LIVMFT]-H-[LIVMF](2)-[PSGA]-

30

[1] Seraphin B. DNA Seq. 3:177-179(1992).

[2] Huang Y., Garrison P.N., Barnes L.D. Biochem. J. 312:925-932(1995).

[3] Barnes L.D., Garrison P.N., Siprashvili Z., Guranowski A., Robinson A.K., Ingram S.W., Croce C.M., Ohta M., Huebner K. Biochemistry 35:11529-11535(1996).

[4] Brenner C., Garrison P., Gilmour J., Peisach D., Ringe D., Petsko G.A., Lowenstein J.M.
Nat. Struct. Biol. 4:231-238(1997).

5 275. Myc-type, 'helix-loop-helix' dimerization domain signature (HLH)

A number of eukaryotic proteins, which probably are sequence specific DNA-binding proteins that act as transcription factors, share a conserved domain of 40 to 50 amino acid residues. It has been proposed [1] that this domain is formed of two amphipathic helices joined by a variable length linker region that could form a loop. This 'helix-loop-helix' (HLH) domain mediates protein dimerization and has been found in the proteins listed below [2,3,E1,E2]. Most of these proteins have an extra basic region of about 15 amino acid residues that is adjacent to the HLH domain and specifically binds to DNA. They are referred as basic helix-loop-helix proteins (bHLH), and are classified in two groups: class A (ubiquitous) and class B (tissue-specific). Members of the bHLH family bind variations on the core sequence 'CANNTG', also referred to as the E-box motif. The homo- or heterodimerization mediated by the HLH domain is independent of, but necessary for DNA binding, as two basic regions are required for DNA binding activity. The HLH proteins lacking the basic domain (Ecm, Id) function as negative regulators since they form heterodimers, but fail to bind DNA. The hairy-related proteins (hairy, E(spl), deadpan) also repress transcription although they can bind DNA. The proteins of this subfamily act together with co-repressor proteins, like groucho, through their C-terminal motif WRPW. - The myc family of cellular oncogenes [4], which is currently known to contain four members: c-myc [E3], N-myc, L-myc, and B-myc. The myc genes are thought to play a role in cellular differentiation and proliferation. - Proteins involved in myogenesis (the induction of muscle cells). In mammals MyoD1 (Myf-3), myogenin (Myf-4), Myf-5, and Myf-6 (Mrf4 or herculin), in birds CMD1 (QMF-1), in Xenopus MyoD and MF25, in Caenorhabditis elegans CeMyoD, and in Drosophila nautilus (nau). - Vertebrate proteins that bind specific DNA sequences ('E boxes') in various immunoglobulin chains enhancers: E2A or ITF-1 (E12/pan-2 and E47/pan-1), ITF-2 (tcf4), TFE3, and TFEB. - Vertebrate neurogenic differentiation factor 1 that acts as differentiation factor during neurogenesis. - Vertebrate MAX protein, a transcription regulator that forms a sequence-specific DNA-binding protein complex with myc or mad. - Vertebrate Max Interacting Protein 1 (MXI1 protein) which acts as a transcriptional repressor and may antagonize myc transcriptional activity by competing for max. - Proteins of the bHLH/PAS superfamily which are transcriptional activators. In

mammals, AH receptor nuclear translocator (ARNT), single-minded homologs (SIM1 and SIM2), hypoxia-inducible factor 1 alpha (HIF1A), AH receptor (AHR), neuronal pas domain proteins (NPAS1 and NPAS2), endothelial pas domain protein 1 (EPAS1), mouse ARNT2, and human BMAL1. In drosophila, single-minded (SIM), AH receptor nuclear translocator (ARNT), tracheless protein (TRH), and similar protein (SIMA). - Mammalian transcription factors HES, which repress transcription by acting on two types of DNA sequences, the E box and the N box. - Mammalian MAD protein (max dimerizer) which acts as transcriptional repressor and may antagonize myc transcriptional activity by competing for max. - Mammalian Upstream Stimulatory Factor 1 and 2 (USF1 and USF2), which bind to a symmetrical DNA sequence that is found in a variety of viral and cellular promoters. - Human lyl-1 protein; which is involved, by chromosomal translocation, in T- cell leukemia. - Human transcription factor AP-4. - Mouse helix-loop-helix proteins MATH-1 and MATH-2 which activate E box- dependent transcription in collaboration with E47. - Mammalian stem cell protein (SCL) (also known as tal1), a protein which may play an important role in hemopoietic differentiation. SCL is involved, by chromosomal translocation, in stem-cell leukemia. - Mammalian proteins Id1 to Id4 [5]. Id (inhibitor of DNA binding) proteins lack a basic DNA-binding domain but are able to form heterodimers with other HLH proteins, thereby inhibiting binding to DNA. - Drosophila extra-macrochaetae (emc) protein, which participates in sensory organ patterning by antagonizing the neurogenic activity of the achaete- scute complex. Emc is the homolog of mammalian Id proteins. - Human Sterol Regulatory Element Binding Protein 1 (SREBP-1), a transcriptional activator that binds to the sterol regulatory element 1 (SRE-1) found in the flanking region of the LDLR gene and in other genes. - Drosophila achaete-scute (AS-C) complex proteins T3 (l'sc), T4 (scute), T5 (achaete) and T8 (asense). The AS-C proteins are involved in the determination of the neuronal precursors in the peripheral nervous system and the central nervous system. - Mammalian homologs of achaete-scute proteins, the MASH-1 and MASH-2 proteins. - Drosophila atonal protein (ato) which is involved in neurogenesis. - Drosophila daughterless (da) protein, which is essential for neurogenesis and sex-determination. - Drosophila deadpan (dpn), a hairy-like protein involved in the functional differentiation of neurons. - Drosophila delilah (dei) protein, which is plays an important role in the differentiation of epidermal cells into muscle. - Drosophila hairy (h) protein, a transcriptional repressor which regulates the embryonic segmentation and adult bristle patterning. - Drosophila enhancer of split proteins E(spl), that are hairy-like proteins active during neurogenesis. also act as transcriptional repressors. - Drosophila twist (twi) protein, which is involved in the establishment of germ

Amphipathic helix 1 Loop Amphipathic helix 2. The signature pattern developed to detect this domain spans completely the second amphipathic helix.

Consensus pattern: [DENSTAP]-[KTR]-[LIVMAGSNT]-{FYWCPHKR}-[LIVMT]-
[LIVM]-x(2)-[STAV]-[LIVMSTACKR]-x-[VMFYH]-[LIVMTA]-{P}-{P}-
[LIVMRKHQ]-

- [1] Murre C., McCaw P.S., Baltimore D. Cell 56:777-783(1989).
- [2] Garrel J., Campuzano S. BioEssays 13:493-498(1991).
- [3] Kato G.J., Dang C.V. FASEB J. 6:3065-3072(1992).
- [4] Krause M., Fire A., Harrison S.W., Priess J., Weintraub H. Cell 63:907-919(1990).
- [5] Riechmann V., van Cruchten I., Sablitzky F. Nucleic Acids Res. 22:749-755(1994).

276. HMG14 and HMG17 signature

High mobility group (HMG) proteins are a family of relatively low molecular weight non-histone components in chromatin. HMG14 and HMG17 [1], two related proteins of about 100 amino acid residues, bind to the inner side of the nucleosomal DNA thus altering the interaction between the DNA and the histone octamer. These two proteins may be involved in the process which maintains transcribable genes in a unique chromatin conformation. The trout nonhistone chromosomal protein H6 (histone T) also belongs to this family. As a signature pattern a conserved stretch of 10 residues located in the N-terminal section of HMG14 and HMG17 was selected.

Consensus pattern: R-R-S-A-R-L-S-A-[RK]-P-

[1] Bustin M., Reeves R. Prog. Nucleic Acid Res. Mol. Biol. 54:35-100(1996).

5

277. Hydroxymethylglutaryl-coenzyme A lyase active site (HMGL1)

3-hydroxy-3-methylglutaryl-coenzyme A lyase (HMG-CoA lyase or HL) (EC

4.1.3.4) catalyzes the transformation of HMG-CoA into acetyl-CoA and acetoacetate. In

vertebrates it is a mitochondrial enzyme which is involved in ketogenesis and in leucine

10 catabolism [1]. In some bacteria, such as *Pseudomonas mevalonii*, it is involved in

mevalonate catabolism (gene *mvaB*). A cysteine has been shown [2], in *mvaB*, to be required for the activity of the enzyme. The region around this residue is perfectly conserved and is used as a signature pattern.

15 Consensus pattern: S-V-A-G-L-G-G-C-P-Y [C is the active site residue]-

[1] Mitchell G.A., Robert M.-F., Hruz P.W., Wang S., Fontaine G., Behnke C.E., Mende-Mueller L.M., Schappert K., Lee C., Gibson K.M., Mizioro H.M. J. Biol. Chem. 268:4376-4381(1993).

20 [2] Hruz P.W., Narasimhan C., Mizioro H.M. Biochemistry 31:6842-6847(1992).

Alpha-isopropylmalate and homocitrate synthases signatures (HMGL2)

The following enzymes have been shown [1] to be functionally as well as evolutionary

related: - Alpha-isopropylmalate synthase (EC 4.1.3.12) which catalyzes the first step in the

25 biosynthesis of leucine, the condensation of acetyl-CoA and alpha- ketoisovalerate to form 2-

isopropylmalate synthase. - Homocitrate synthase (EC 4.1.3.21) (gene *nifV*) which is

involved in the biosynthesis of the iron-molybdenum cofactor of nitrogenase and catalyzes

the condensation of acetyl-CoA and alpha-ketoglutarate into homocitrate. - Soybean late

nodulin 56. - *Methanococcus jannaschii* hypothetical proteins MJ0503, MJ1195 and MJ1392.

30 Two conserved regions were selected as signature patterns for these enzymes. The first region is located in the N-terminal section while the second region is located in the central section and contains two conserved histidine residues which could be implicated in the catalytic mechanism.

Consensus pattern: L-R-[DE]-G-x-Q-x(10)-K-

Consensus pattern: [LIVMFW]-x(2)-H-x-H-[DN]-D-x-G-x-[GAS]-x-[GASLI]-

[1] Wang S.-Z., Dean D.R., Chen J.-S., Johnson J.L. J. Bacteriol. 173:3041-3046(1991).

5

278. (HMG CoA synt) Hydroxymethylglutaryl-coenzyme A synthase active site
Hydroxymethylglutaryl-coenzyme A synthase (EC 4.1.3.5) (HMG-CoA synthase) catalyzes
the condensation of acetyl-CoA with acetoacetyl-CoA to produce HMG- CoA and CoA [1].In
vertebrates there are two isozymes located in different subcellular compartments: a cytosolic
form which is the starting point of the mevalonate pathway which leads to cholesterol and
other sterolic and isoprenoid compounds and a mitochondrial form responsible for ketone
body biosynthesis. HMG-CoA is also found in other eukaryotes such as insect, plants and
fungi. A cysteine is known to act as the catalytic nucleophile in the first step of the reaction,
the acetylation of the enzyme by acetyl-CoA. The conserved region was used around this
active site residue as a signature pattern.

Consensus pattern: N-x-[DN]-[IV]-E-G-[IV]-D-x(2)-N-A-C-[FY]-x-G [C is the active site
residue]-

[1] Rokosz L.L., Boulton D.A., Butkiewicz E.A., Sanyal G., Cueto M.A., Lachance P.A.,
Hermes J.D. Arch. Biochem. Biophys. 312:1-13(1994).

279. HMG (high mobility group) box

280. HSF-type DNA-binding domain signature

Heat shock factor (HSF) is a DNA-binding protein that specifically binds heat shock
promoter elements (HSE). HSE is a palindromic element rich with repetitive purine and
pyrimidine motifs: 5'-nGAAnnTTCnnGAAnnTTCn-3'. HSF is expressed at normal
temperatures but is activated by heat shock or chemical stressors [1,2]. The sequences of HSF
from various species show extensive similarity in a region of about 90 amino acids, which
has been shown [3] to bind DNA. Some other proteins also contain a HSF domain, these are:

- Yeast SFL1, a protein involved in cell surface assembly and regulation of the gene related to flocculation (asexual cell aggregation) [4]. - Yeast transcription factor SKN7 (or BRY1 or POS9), which binds to the promoter elements SCB and MCB essential for the control of G1 cyclins expression [5]. - Yeast MGA1. - Yeast hypothetical protein YJR147w. A pattern from the most conserved part of the HSF DNA-binding domain was derived, its central region.

Consensus pattern: L-x(3)-[FY]-K-H-x-N-x-[STAN]-S-F-[LIVM]-R-Q-L-[NH]-x-Y-x-[FYW]-[RKH]-K-[LIVM]-

- [1] Sorger P.K. *Cell* 65:363-366(1991).
 [2] Mager W.H., Moradas Ferreira P. *Biochem. J.* 290:1-13(1993).
 [3] Vuister G.W., Kim S.-J., Orosz A., Marquardt J., Wu C., Bax A. *Nat. Struct. Biol.* 1:605-613(1994).
 [4] Fujita A., Kikuchi Y., Kuhara S., Misumi Y., Matsumoto S., Kobayashi H. *Gene* 85:321-328(1989).
 [5] Morgan B.A., Bouquin N., Merrill G.F., Johnston L.H. *EMBO J.* 14:5679-5689(1995).

281. Heat shock hsp20 proteins family profile

- Prokaryotic and eukaryotic organisms respond to heat shock or other environmental stress by inducing the synthesis of proteins collectively known as heat-shock proteins (hsp) [1]. Amongst them is a family of proteins with an average molecular weight of 20 Kd, known as the hsp20 proteins [2 to 5]. These seem to act as chaperones that can protect other proteins against heat-induced denaturation and aggregation. Hsp20 proteins seem to form large heterooligomeric aggregates; their family is currently composed of the following members: - Vertebrate heat shock protein hsp27 (hsp25), induced by a variety of environmental stresses. - *Drosophila* heat shock proteins hsp22, hsp23, hsp26, hsp27, hsp67BA and BC. - *Caenorhabditis elegans* hsp16 multigene family. - Fungal HSP26 (budding yeast) and hsp30 (*Neurospora crassa* and *Aspergillus nidulans*). - Plant small hsp's. Plants have four classes of hsp20: classes I and II which are cytoplasmic, class III which is chloroplastic and class IV which is found in the endomembrane. - Alpha-crystallin A and B chains. Alpha-crystallin is an abundant constituent of the eye lens of most vertebrate species. Its main function appears to be to maintain the correct refractive index of the lens. It is also found in other tissues where it seems to act as a chaperone [6]. - *Schistosoma mansoni* major egg antigen p40.

Structurally, p40 is built of two tandem hsp20 domains. - A variety of prokaryotic proteins: ibpA and ibpB from *Escherichia coli*, hsp18 from *Clostridium acetobutylicum*, spore protein SP21 (hspA) from *Stigmatella aurantiaca*, *Mycobacterium leprae* 18 Kd antigen and *Mycobacterium tuberculosis* 14 Kd antigen. - *Methanococcus jannaschii* hypothetical protein MJ0285. Structurally, this family is characterized by the presence of a conserved C-terminal domain of about 100 residues. The profile developed to detect members of the hsp20 family is based on an alignment of this domain.

-Sequences known to belong to this class detected by the profile: ALL.

[1] Lindquist S., Craig E.A. *Annu. Rev. Genet.* 22:631-677(1988).[2] de Jong W.W., Leunissen J.A.M., Voorter C.E.M. *Mol. Biol. Evol.* 10:103-126(1993).[3] Caspers G.J., Leunissen J.A.M., de Jong W.W. *J. Mol. Evol.* 40:238-248(1995).[4] Jaenicke R., Creighton T.E. *Curr. Biol.* 3:234-235(1993).[5] Jakob U., Buchner J. *Trends Biochem. Sci.* 19:205-211(1994).[6] Groenen P.J.T.A., Merck K.B., de Jong W.W., Bloemendal H. *Eur. J. Biochem.* 225:1-9(1994).

282. Heat shock hsp70 proteins family signatures

Prokaryotic and eukaryotic organisms respond to heat shock or other environmental stress by the induction of the synthesis of proteins collectively known as heat-shock proteins (hsp) [1]. Amongst them is a family of proteins with an average molecular weight of 70 Kd, known as the hsp70proteins [2,3,4]. In most species, there are many proteins that belong to the hsp70 family. Some of them are expressed under unstressed conditions. Hsp70proteins can be found in different cellular compartments (nuclear, cytosolic, mitochondrial, endoplasmic reticulum, etc.). Some of the hsp70 family proteins are listed below: - In *Escherichia coli* and other bacteria, the main hsp70 protein is known as the dnaK protein. A second protein, hscA, has been recently discovered. dnaK is also found in the chloroplast genome of red algae. - In yeast, at least ten hsp70 proteins are known to exist: SSA1 to SSA4, SSB1, SSB2, SSC1, SSD1 (KAR2), SSE1 (MSI3) and SSE2. - In *Drosophila*, there are at least eight different hsp70 proteins: HSP70, HSP68, and HSC-1 to HSC-6. - In mammals, there are at least eight different proteins: HSPA1 to HSPA6, HSC70, and GRP78 (also known as the immunoglobulin heavy chain binding protein (BiP)). - In the sugar beet yellow virus (SBYV), a hsp70 homolog has been shown [5] to exist. - In archaeobacteria, hsp70 proteins are also present [6]. All proteins belonging to the hsp70 family bind ATP. A variety of functions has been postulated for hsp70 proteins. It now appears [7] that some hsp70proteins

play an important role in the transport of proteins across membranes. They also seem to be involved in protein folding and in the assembly/disassembly of protein complexes [8]. Three signature patterns for the hsp70 family of proteins were derived; the first centered on a conserved pentapeptide found in the N-terminal section of these proteins; the two others on conserved regions located in the central part of the sequence.

Consensus pattern: [IV]-D-L-G-T-[ST]-x-[SC] -

Consensus pattern: [LIVMF]-[LIVMFY]-[DN]-[LIVMFS]-G-[GSH]-[GS]-[AST]-x(3)-[ST]-[LIVM]-[LIVMFC]-

Consensus pattern: [LIVMY]-x-[LIVMF]-x-G-G-x-[ST]-x-[LIVM]-P-x-[LIVM]-x-[DEQKRSTA]-

[1] Lindquist S., Craig E.A. Annu. Rev. Genet. 22:631-677(1988).

[2] Pelham H.R.B. Cell 46:959-961(1986).

[3] Pelham H.R.B. Nature 332:776-77(1988), [4] Craig E.A. BioEssays 11:48-52(1989).

[5] Agranovsky A.A., Boyko V.P., Karasev A.V., Koonin E.V., Dolja V.V. J. Mol. Biol. 217:603-610(1991).

[6] Gupta R.S., Singh B. J. Bacteriol. 174:4594-4605(1992).

[7] Deshaies R.J., Koch B.D., Schekman R. Trends Biochem. Sci. 13:384-388(1988).

[8] Craig E.A., Gross C.A. Trends Biochem. Sci. 16:135-140(1991).

283. Heat shock hsp90 proteins family signature

Prokaryotic and eukaryotic organisms respond to heat shock or other environmental stress by the induction of the synthesis of proteins collectively known as heat-shock proteins (hsp) [1]. Amongst them is a family of proteins, with an average molecular weight of 90 Kd, known as the hsp90 proteins. Proteins known to belong to this family are: - Escherichia coli and other bacteria heat shock protein c62.5 (gene htpG). - Vertebrate hsp 90-alpha (hsp 86) and hsp 90-beta (hsp 84). - Drosophila hsp 82 (hsp 83). - Trypanosoma cruzi hsp 85. - Plants Hsp82 or Hsp83. - Yeast and other fungi HSC82, and HSP82. - The endoplasmic reticulum protein 'endoplasmic' (also known as Erp99 in mouse, GRP94 in hamster, and hsp 108 in chicken). The exact function of hsp90 proteins is not yet known. In higher eukaryotes, hsp90 has been found associated with steroid hormone receptors, with tyrosine kinase oncogene products of several retroviruses, with eIF2alpha kinase, and with actin and tubulin. Hsp90 are

probable chaperonins that possess ATPase activity [2,3]. As a signature pattern for the hsp90 family of proteins, a highly conserved region found in the N-terminal part of these proteins was selected.

5 Consensus pattern: Y-x-[NQH]-K-[DE]-[IVA]-F-L-R-[ED] -

[1] Lindquist S., Craig E.A. Annu. Rev. Genet. 22:631-677(1988).

[2] Nadeau K., Das A., Walsh C.T. J. Biol. Chem. 268:1479-1487(1993).

[3] Jakob U., Buchner J. Trends Biochem. Sci. 19:205-211(1994).

10

284. Helix-turn-helix (HTH3)

This large family of DNA binding helix-turn helix proteins includes Cro

Swiss:P03036 and CI Swiss:P03034.

15

285. Heme oxygenase signature

Heme oxygenase (EC 1.14.99.3) (HO) [1] is the microsomal enzyme that, in animals, carries out the oxidation of heme, it cleaves the heme ring at the alpha methene bridge to form

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biliverdin and carbon monoxide. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. In mammals there are three isozymes of heme oxygenase: HO-1 to HO-

3. The first two isozymes differ in their tissue expression and their inducibility: HO-1 is highly inducible by its substrate heme and by various non-heme substances, while HO-2 is

25

non-inducible. It has been suggested [2] that HO-2 could be implicated in the production of

30

carbon monoxide in the brain where it is said to act as a neurotransmitter. In the genome of the chloroplast of red algae as well as in cyanobacteria, there is a heme oxygenase (gene pbsA) that is the key enzyme in the synthesis of the chromophoric part of the photosynthetic antennae [3]. A heme oxygenase is also present in the bacteria *Corynebacterium diphtheriae* (gene hmuO), where it is involved in the acquisition of iron from the host heme [4]. There is,

35

in the central section of these enzymes, a well conserved region centered on a histidine residue which is proposed to play a key role in binding the substrate heme at the active center of the enzyme. This region was used as a signature pattern.

Consensus pattern: L-[IV]-A-H-[STACH]-Y-[STV]-[RT]-Y-[LIVM]-G [H binds the heme] -

- [1] Maines M.D. FASEB J. 2:2557-2568(1988).
 [2] Barinaga M. Science 259:309-309(1993).
 [3] Richaud C., Zabulon G. Proc. Natl. Acad. Sci. U.S.A. 94:11736-11741(1997).
 5 [4] Schmitt M.P. J. Bacteriol. 179:838-845(1997).

286. Hepatitis core antigen.

10 The core antigen of hepatitis viruses possesses a carboxyl terminus rich in arginine. On this basis it was predicted that the core antigen would bind DNA [1]. There is some experimental evidence to support this [2].

- 15 [1] Pasek M, Goto T, Gilbert W, Zink B, Schaller H, McKay P, Leadbetter G, Murray K; Nature 1979;282:575-579. [2]
 Gallina A, Bonelli F, Zentilin L, Rindi G, Muttini M, Milanese G; J Virol 1989;63:4645-4652.

20 287. Histidine biosynthesis protein

Proteins involved in steps 4 and 6 of the histidine biosynthesis pathway are contained in this family. Histidine is formed by several complex and distinct biochemical reactions catalysed by eight enzymes. The enzymes in this Pfam entry are called His6 and His7 in
 25 eukaryotes and HisA and HisF in prokaryotes.

- [1] Fani R, Tamburini E, Mori E, Lazcano A, Lio P, Barberio C, Casalone E, Cavalieri D, Perito B, Polsinelli M, Gene 1997;197:9-17. [2] Fani R, Lio P, Chiarelli I, Bazzicalupo M, J Mol Evol 1994;38:489-495.

30 288. Histone deacetylase family

Histones can be reversibly acetylated on several lysine residues. Regulation of transcription is caused in part by this mechanism. Histone deacetylases catalyse the removal of the acetyl group. Histone deacetylases are related to other proteins [1].

289. Histidinol dehydrogenase signature

- 5 Histidinol dehydrogenase (EC 1.1.1.23) (HDH) catalyzes the terminal step in the biosynthesis of histidine in bacteria, fungi, and plants, the four-electron oxidation of L-histidinol to histidine. In bacteria HDH is a single chain polypeptide; in fungi it is the C-terminal domain of a multifunctional enzyme which catalyzes three different steps of histidine biosynthesis; and in plants it is expressed as nuclear encoded protein precursor which is exported to the
- 10 chloroplast [1]. As a signature pattern a highly conserved region located in the central part of HDH was selected. This region does not correspond to the part of the enzyme that, in most, but not all HDH sequences contains a cysteine residue which, in *Salmonella typhimurium*, has been said [2] to be important for the catalytic activity of the enzyme.
- 15 Consensus pattern: I-D-x(2)-A-G-P-[ST]-E-[LIVS]-[LIVMA](3)-[AC]-x(3)-A-x(4)-[LIVM]-[AV]-[SACL]-[DE]-[LIVMFC]-[LIVM]-[SA]-x(2)-E-H-
- [1] Nagai A., Ward E., Beck J., Tada S., Chang J.-Y., Scheidegger A., Ryals J. Proc. Natl. Acad. Sci. U.S.A. 88:4133-4137(1991).
- 20 [2] Grubmeyer C.T., Gray W.R. Biochemistry 25:4778-4784(1986).

290. Homoserine dehydrogenase signature

- Homoserine dehydrogenase (EC 1.1.1.3) (HDh) [1,2] catalyzes NAD-dependent reduction of
- 25 aspartate beta-semialdehyde into homoserine. This reaction is the third step in a pathway leading from aspartate to homoserine. The latter participates in the biosynthesis of threonine and then isoleucine as well as in that of methionine. HDh is found either as a single chain protein as in some bacteria and yeast, or as a bifunctional enzyme consisting of an N-terminal aspartokinase domain and a C-terminal HDh domain as in bacteria such as *Escherichia coli*
- 30 and in plants. As a signature pattern, the best conserved region of Hdh has been selected. This is a segment of 23 to 24 residues located in the central section and that contains two conserved aspartate residues.

Consensus pattern: A-x(3)-G-[LIVMFY]-[STAG]-x(2,3)-[DNS]-P-x(2)-D-[LIVM]-x-G- x-D-x(3)-K-

[1] Thomas D., Barbey R., Surdin-Kerjan Y. FEBS Lett. 323:289-293(1993).

5 [2] Cami B., Clepet C., Patte J.-C. Biochimie 75:487-495(1993).

291. haloacid dehalogenase-like hydrolase

This family is structurally different from the alpha/ beta hydrolase family (abhydrolase). This family includes L-2-haloacid dehalogenase, epoxide hydrolases and phosphatases. The structure of the family consists of two domains. One is an inserted four helix bundle, which is the least well conserved region of the alignment, between residues 16 and 96 of Swiss:P24069. The rest of the fold is composed of the core alpha/beta domain.

[1] Hisano T, Hata Y, Fujii T, Liu JQ, Kurihara T, Esaki N, Soda K, J Biol Chem 1996; 15 271:20322-20330.

292. DEAD and DEAH box families ATP-dependent helicases signatures (helicase_C)

A number of eukaryotic and prokaryotic proteins have been characterized [1,2,3] on the basis of their structural similarity. They all seem to be involved in ATP-dependent, nucleic-acid unwinding. Proteins currently known to belong to this family are: - Initiation factor eIF-4A. Found in eukaryotes, this protein is a subunit of a high molecular weight complex involved in 5'cap recognition and the binding of mRNA to ribosomes. It is an ATP-dependent RNA-helicase. - PRP5 and PRP28. These yeast proteins are involved in various ATP-requiring steps of the pre-mRNA splicing process. - P110, a mouse protein expressed specifically during spermatogenesis. - An3, a Xenopus putative RNA helicase, closely related to P110. - SPP81/DED1 and DBP1, two yeast proteins probably involved in pre-mRNA splicing and related to P110. - Caenorhabditis elegans helicase glh-1. - MSS116, a yeast protein required for mitochondrial splicing. - SPB4, a yeast protein involved in the maturation of 25S ribosomal RNA. - p68, a human nuclear antigen. p68 has ATPase and DNA-helicase activities in vitro. It is involved in cell growth and division. - Rm62 (p62), a Drosophila putative RNA helicase related to p68. - DBP2, a yeast protein related to p68. - DHH1, a yeast protein. - DRS1, a yeast protein involved in ribosome assembly. - MAK5, a yeast protein involved in maintenance of dsRNA killer plasmid. - ROK1, a yeast protein. - ste13, a fission

yeast protein. - Vasa, a Drosophila protein important for oocyte formation and specification of embryonic posterior structures. - Me31B, a Drosophila maternally expressed protein of unknown function. - dbpA, an Escherichia coli putative RNA helicase. - deaD, an Escherichia coli putative RNA helicase which can suppress a mutation in the rpsB gene for ribosomal protein S2. - rhlB, an Escherichia coli putative RNA helicase. - rhlE, an Escherichia coli putative RNA helicase. - srmB, an Escherichia coli protein that shows RNA-dependent ATPase activity. It probably interacts with 23S ribosomal RNA. - Caenorhabditis elegans hypothetical proteins T26G10.1, ZK512.2 and ZK686.2. - Yeast hypothetical protein YHR065c. - Yeast hypothetical protein YHR169w. - Fission yeast hypothetical protein SpAC31A2.07c. - Bacillus subtilis hypothetical protein yxiN. All these proteins share a number of conserved sequence motifs. Some of them are specific to this family while others are shared by other ATP-binding proteins or by proteins belonging to the helicases 'superfamily' [4,E1]. One of these motifs, called the 'D-E-A-D-box', represents a special version of the B motif of ATP-binding proteins. Some other proteins belong to a subfamily which have His instead of the second Asp and are thus said to be 'D-E-A-H-box' proteins [3,5,6,E1]. Proteins currently known to belong to this subfamily are: - PRP2, PRP16, PRP22 and PRP43. These yeast proteins are all involved in various ATP-requiring steps of the pre-mRNA splicing process. - Fission yeast prh1, which may be involved in pre-mRNA splicing. - Male-less (mle), a Drosophila protein required in males, for dosage compensation of X chromosome linked genes. - RAD3 from yeast. RAD3 is a DNA helicase involved in excision repair of DNA damaged by UV light, bulky adducts or cross-linking agents. Fission yeast rad15 (rhp3) and mammalian DNA excision repair protein XPD (ERCC-2) are the homologs of RAD3. - Yeast CHL1 (or CTF1), which is important for chromosome transmission and normal cell cycle progression in G(2)/M. - Yeast TPS1. - Yeast hypothetical protein YKL078w. - Caenorhabditis elegans hypothetical proteins C06E1.10 and K03H1.2. - Poxviruses' early transcription factor 70 Kd subunit which acts with RNA polymerase to initiate transcription from early gene promoters. - I8, a putative vaccinia virus helicase. - hrpA, an Escherichia coli putative RNA helicase. Signature patterns were developed for both subfamilies.

Consensus pattern: [LIVMF](2)-D-E-A-D-[RKEN]-x-[LIVMFYGSTN]-

Consensus pattern: [GSAH]-x-[LIVMF](3)-D-E-[ALIV]-H-[NECR] -

Note: proteins belonging to this family also contain a copy of the ATP/GTP- binding motif 'A' (P-loop) (see the relevant entry <PDOC00017

- [1] Schmid S.R., Linder P. Mol. Microbiol. 6:283-292(1992).
[2] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).
5 [3] Wassarman D.A., Steitz J.A. Nature 349:463-464(1991).
[4] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).
[5] Harosh I., Deschavanne P. Nucleic Acids Res. 19:6331-6331(1991).
[6] Koonin E.V., Senkevich T.G. J. Gen. Virol. 73:989-993(1992).

10 293. Heme-binding domain in cytochrome b5 and oxidoreductases (heme_1)

Cytochrome b5 is a membrane-bound hemo protein which acts as an electron carrier for several membrane-bound oxygenases [1]. There are two homologous forms of b5, one found in microsomes and one found in the outer membrane of mitochondria. Two conserved
15 histidine residues serve as axial ligands for the heme group. The structure of a number of oxidoreductases consists of the juxtaposition of a heme-binding domain homologous to that of b5 and either a flavodehydrogenase or a molybdopterin domain. These enzymes are:

- Lactate dehydrogenase (EC 1.1.2.3) [2], an enzyme that consists of a flavodehydrogenase domain and a heme-binding domain called cytochrome b2.
- 20 - Nitrate reductase (EC 1.6.6.1), a key enzyme involved in the first step of nitrate assimilation in plants, fungi and bacteria [3,4]. Consists of a molybdopterin domain (see <PDOC00484>), a heme-binding domain called cytochrome b557, as well as a cytochrome reductase domain.
- Sulfite oxidase (EC 1.8.3.1) [5], which catalyzes the terminal reaction in the
25 oxidative degradation of sulfur-containing amino acids. Also consists of a molybdopterin domain and a heme-binding domain.

This family of proteins also includes:

- TU-36B, a Drosophila muscle protein of unknown function [6].
- Fission yeast hypothetical protein SpAC1F12.10c.
- 30 - Yeast hypothetical protein YMR073c.
- Yeast hypothetical protein YMR272c.

A segment was used which includes the first of the two histidine heme ligands, as a signature pattern for the heme-binding domain of cytochrome b5 family.

Consensus pattern: [FY]-[LIVMK]-x(2)-H-P-[GA]-G [H is a heme axial ligand]-

[1] Ozols J. *Biochim. Biophys. Acta* 997:121-130(1989).

[2] Guiard B. *EMBO J.* 4:3265-3272(1985).

5 [3] Calza R., Huttner E., Vincentz M., Rouze P., Galangau F., Vaucheret H., Cherel I., Meyer C., Kronenberger J., Caboche M. *Mol. Gen. Genet.* 209:552-562(1987).

[4] Crawford N.M., Smith M., Bellissimo D., Davis R.W. *Proc. Natl. Acad. Sci. U.S.A.* 85:5006-5010(1988).

[5] Guiard B., Lederer F. *Eur. J. Biochem.* 100:441-453(1979).

10 [6] Levin R.J., Boychuk P.L., Croniger C.M., Kazzaz J.A., Rozek C.E. *Nucleic Acids Res.* 17:6349-6367(1989).

294. Hexapeptide-repeat containing-transferases signature

- 15 On the basis of sequence similarity, a number of transferases have been proposed [1,2,3,4] to belong to a single family. These proteins are: - Serine acetyltransferase (EC 2.3.1.30) (SAT) (gene *cysE*), an enzyme involved in cysteine biosynthesis. - *Azotobacter chroococcum* nitrogen fixation protein *nifP*. *NifP* is most probably a SAT involved in the optimization of nitrogenase activity. - *Escherichia coli* thiogalactoside acetyltransferase (EC 2.3.1.18) (gene
- 20 *lacA*), an enzyme involved in the biosynthesis of lactose. - UDP-N-acetylglucosamine acyltransferase (EC 2.3.1.129) (gene *lpxA*), an enzyme involved in the biosynthesis of lipid A, a phosphorylated glycolipid that anchors the lipopolysaccharide to the outer membrane of the cell. - UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase (EC 2.3.1.-) (gene *lpxD* or *firA*), which is also involved in the biosynthesis of lipid A. - Chloramphenicol
- 25 acetyltransferase (CAT) (EC 2.3.1.28) from *Agrobacterium tumefaciens*, *Bacillus sphaericus*, *Escherichia coli* plasmid IncFII NR79, *Pseudomonas aeruginosa*, *Staphylococcus aureus* plasmid pIP630. These CAT are not evolutionary related to the main family of CAT (see <PDOC00093>). - *Rhizobium* nodulation protein *nodL*. *NodL* is an acetyltransferase involved in the O-acetylation of Nod factors. - Bacterial maltose O-acetyltransferase (EC
- 30 2.3.1.79). - Bacterial tetrahydronicotinate N-succinyltransferase (EC 2.3.1.117) (gene *dapD*) which catalyzes the fourth step in the biosynthesis of diaminopimelate and lysine from aspartate semialdehyde. - Bacterial N-acetylglucosamine-1-phosphate uridylyltransferase (EC 2.7.7.23) (gene *glmU* or *gcaD* or *tms*), an enzyme involved in peptidoglycan and lipopolysaccharide biosynthesis. - *Staphylococcus aureus* protein *capG* which is involved in

biosynthesis of type 1 capsular polysaccharide. - Yeast hypothetical protein YJL218w, which is highly similar to *Escherichia coli* lacA. - Fission yeast hypothetical protein SpAC18B11.09c. - *Methanococcus jannaschii* hypothetical protein MJ1064. These proteins have been shown [3,4] to contain a repeat structure composed of tandem repeats of a [LIV]-G-x(4) hexapeptide which, in the tertiary structure of lpxA [5], has been shown to form a left-handed parallel beta helix. Our signature pattern is based on a fourfold repeat of this hexapeptide.

Consensus pattern: [LIV]-[GAED]-x(2)-[STAV]-x-[LIV]-x(3)-[LIVAC]-x-[LIV]-[GAED]-x(2)-[STAVR]-x-[LIV]-[GAED]-x(2)-[STAV]-x-[LIV]-x(3)-[LIV]-

[1] Downie J.A. Mol. Microbiol. 3:1649-1651(1989).

[2] Parent R., Roy P.H. J. Bacteriol. 174:2891-2897(1992).

[3] Vaara M. FEMS Microbiol. Lett. 97:249-254(1992).

[4] Vuorio R., Haerkonen T., Tolvanen M., Vaara M. FEBS Lett. 337:289-292(1994).

[5] Ractz C.R.H., Roderick S.L. Science 270:997-1000(1995).

295. Hexokinases signature. Hexokinase (EC 2.7.1.1) [1,2] is an important glycolytic enzyme that catalyzes the phosphorylation of keto- and aldohexoses (e.g. glucose, mannose and fructose) using MgATP as the phosphoryl donor. In vertebrates there are four major isoenzymes, commonly referred as types I, II, III and IV. Type IV hexokinase, which is often incorrectly designated glucokinase [3], is only expressed in liver and pancreatic beta-cells and plays an important role in modulating insulin secretion; it is a protein of a molecular mass of about 50 Kd. Hexokinases of types I to III, which have low Km values for glucose, have a molecular mass of about 100 Kd. Structurally they consist of a very small N-terminal hydrophobic membrane-binding domain followed by two highly similar domains of 450 residues. The first domain has lost its catalytic activity and has evolved into a regulatory domain. In yeast there are three different isozymes: hexokinase PI (gene HXK1), PII (gene HXKB), and glucokinase (gene GLK1). All three proteins have a molecular mass of about 50 Kd. All these enzymes contain one (or two in the case of types I to III isozymes) strongly conserved region which has been shown [4] to be involved in substrate binding. A pattern from that region has been derived

Consensus pattern: [LIVM]-G-F-[TN]-F-S-[FY]-P-x(5)-[LIVM]-[DNST]-x(3)-[LIVM]-x(2)-
W-T-K-x-[LF]-

[1] Middleton R.J. Biochem. Soc. Trans. 18:180-183(1990).[2] Griffin L.D., Gelb B.D.,
5 Wheeler D.A., Davison D., Adams V., McCabe E.R. Genomics 11:1014-1024(1991).[3]
Cornish-Bowden A., Luz Cardenas M. Trends Biochem. Sci. 16:281-282(1991).[4] Schirch
D.M., Wilson J.E. Arch. Biochem. Biophys. 254:385-396(1987).

296. Histone H2A signature (his1)

Histone H2A is one of the four histones, along with H2B, H3 and H4, which forms the
eukaryotic nucleosome core. Using alignments of histone H2A sequences [1,2,E1] as a
signature pattern, a conserved region in the N-terminal part of H2A. This region is conserved
both in classical S-phase regulated H2A's and in variant histone H2A's which are synthesized
15 throughout the cell cycle.

Consensus pattern: [AC]-G-L-x-F-P-V-

[1] Wells D.E., Brown D. Nucleic Acids Res. 19:2173-2188(1991).
20 [2] Thatcher T.H., Gorovsky M.A. Nucleic Acids Res. 22:174-179(1994).

Histone H4 signature (his2)

Histone H4 is one of the four histones, along with H2A, H2B and H3, which forms
the eukaryotic nucleosome core. Along with H3, it plays a central role in nucleosome
25 formation. The sequence of histone H4 has remained almost invariant in more than 2 billion
years of evolution [1,E1]. The region used as a signature pattern is a pentapeptide found in
positions 14 to 18 of all H4 sequences. It contains a lysine residue which is often acetylated
[2] and a histidine residue which is implicated in DNA-binding [3].

30 Consensus pattern: G-A-K-R-H-

[1] Thatcher T.H., Gorovsky M.A. Nucleic Acids Res. 22:174-179(1994).
[2] Doenecke D., Gallwitz D. Mol. Cell. Biochem. 44:113-128(1982).
[3] Ebralidse K.K., Grachev S.A., Mirzabekov A.D. Nature 331:365-367(1988).

Histone H3 signatures (his3)

Histone H3 is one of the four histones, along with H2A, H2B and H4, which forms the eukaryotic nucleosome core. It is a highly conserved protein of 135 amino acid residues [1,2,E1]. The following proteins have been found to contain a C-terminal H3-like domain: - Mammalian centromeric protein CENP-A [3]. Could act as a core histone necessary for the assembly of centromeres. - Yeast chromatin-associated protein CSE4 [4]. - *Caenorhabditis elegans* chromosome III encodes two highly related proteins (F54C8.2 and F58A4.3) whose C-terminal section is evolutionary related to the last 100 residues of H3. The function of these proteins is not yet known. Two signature patterns were developed. The first one corresponds to a perfectly conserved heptapeptide in the N-terminal part of H3. The second one is derived from a conserved region in the central section of H3.

Consensus pattern: K-A-P-R-K-Q-L-

Consensus pattern: P-F-x-[RA]-L-[VA]-[KRQ]-[DEG]-[IV]-

[1] Wells D.E., Brown D. *Nucleic Acids Res.* 19:2173-2188(1991).

[2] Thatcher T.H., Gorovsky M.A. *Nucleic Acids Res.* 22:174-179(1994).

[3] Sullivan K.F., Hechenberger M., Masri K. J. *Cell Biol.* 127:581-592(1994).

[4] Stoler S., Keith K.C., Curnick K.E., Fitzgerald-Hayes M. *Genes Dev.* 9:573-586(1995).

Histone H2B signature (his4)

Histone H2B is one of the four histones, along with H2A, H3 and H4, which forms the eukaryotic nucleosome core. Using alignments of histone H2B sequences [1,2,E1], a conserved region was selected in the C-terminal part of H2B.

Consensus pattern: [KR]-E-[LIVM]-[EQ]-T-x(2)-[KR]-x-[LIVM](2)-x-[PAG]-[DE]-L- x-[KR]-H-A-[LIVM]-[STA]-E-G-

[1] Wells D.E., Brown D. *Nucleic Acids Res.* 19:2173-2188(1991).

[2] Thatcher T.H., Gorovsky M.A. *Nucleic Acids Res.* 22:174-179(1994).

The 'homeobox' is a protein domain of 60 amino acids [1 to 5, E1] first identified in a number of *Drosophila* homeotic and segmentation proteins. It has since been found to be extremely well conserved in many other animals, including vertebrates. This domain binds DNA through a helix-turn-helix type of structure. Some of the proteins which contain a homeobox domain play an important role in development. Most of these proteins are known to be sequence specific DNA-binding transcription factors. The homeobox domain has also been found to be very similar to a region of the yeast mating type proteins. These are sequence-specific DNA-binding proteins that act as master switches in yeast differentiation by controlling gene expression in a cell type-specific fashion. A schematic representation of the homeobox domain is shown below. The helix-turn-helix region is shown by the symbols 'H' (for helix), and 't' (for turn).

xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxHHHHHHHHHttHHHHHHHHHxxxxxxxxx ||||| 1
10 20 30 40 50 60 The pattern to detect homeobox sequences that was developed is 24
residues long and spans positions 34 to 57 of the homeobox domain.

Consensus pattern: [LIVMFYGY]-[ASLVR]-x(2)-[LIVMSTACN]-x-[LIVM]-x(4)-[LIV]-
[RKNEQSTAIY]-[LIVFSTNKH]-W-[FYVC]-x-[NDQTAH]-x(5)-[RKNAIMW]-

[1] Gehring W.J. (In) Guidebook to the homebox genes, Duboule D., Ed., pp1-10, Oxford University Press, Oxford, (1994).

[2] Buerklin T.R. (In) Guidebook to the homebox genes, Duboule D., Ed., pp25-72, Oxford University Press, Oxford, (1994).

[3] Gehring W.J. Trends Biochem. Sci. 17:277-280(1992).

[4] Gehring W.J., Hiromi Y. Annu. Rev. Genet. 20:147-173(1986).

[5] Schofield P.N. Trends Neurosci. 10:3-6(1987).

'Homeobox' antennapedia-type protein signature (home2)

The homeotic Hox proteins are sequence-specific transcription factors. They are part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior (A-P) axis [1]. The hox proteins contain a 'homeobox' domain. In *Drosophila* and other insects, there are eight different Hox genes that are encoded in two gene complexes, ANT-C and BX-C. In vertebrates there are 38 genes organized in four complexes. In six of the eight *Drosophila* Hox genes the homeobox domain is highly similar and a conserved hexapeptide is found five to sixteen amino acids upstream of the homeobox

domain. The six *Drosophila* proteins that belong to this group are antennapedia (Antp), abdominal-A (abd-A), deformed (Dfd), proboscipedia (pb), sex combs reduced (scr) and ultrabithorax (ubx) and are collectively known as the 'antennapedia' subfamily. In vertebrates the corresponding Hox genes are known [2] as Hox-A2, A3, A4, A5, A6, A7, Hox-B1, B2, B3, B4, B5, B6, B7, B8, Hox-C4, C5, C6, C8, Hox-D1, D3, D4 and D8. *Caenorhabditis elegans* lin-39 and mab-5 are also members of the 'antennapedia' subfamily. As a signature pattern for this subfamily of homeobox proteins, the conserved hexapeptide was used.

Consensus pattern: [LIVMF]-[FY]-P-W-M-[KQTA]-

[1] McGinnis W., Krumlauf R. Cell 68:283-302(1992).

[2] Scott M.P. Cell 71:551-553(1992).

'Homeobox' engrailed-type protein signature (home3)

Most proteins which contain a 'homeobox' domain can be classified [1,2], on the basis of their sequence characteristics, in three subfamilies: engrailed, antennapedia and paired. Proteins currently known to belong to the engrailed subfamily are: - *Drosophila* segmentation polarity protein engrailed (en) which specifies the body segmentation pattern and is required for the development of the central nervous system. - *Drosophila* invected protein (inv). - Silk moth proteins engrailed and invected, which may be involved in the compartmentalization of the silk gland. - Honeybee E30 and E60. - Grasshopper (*Schistocerca americana*) G-En. - Mammalian and birds En-1 and En-2. - Zebrafish Eng-1, -2 and -3. - Sea urchin (*Tripneustes gratilla*) SU-HB-en. - Leech (*Helobdella triserialis*) Ht-En. - *Caenorhabditis elegans* ceh-16. Engrailed homeobox proteins are characterized by the presence of a conserved region of some 20 amino-acid residues located at the C-terminal of the 'homeobox' domain. As a signature pattern for this subfamily of proteins, a stretch of eight perfectly conserved residues in this region was used.

Consensus pattern: L-M-A-[EQ]-G-L-Y-N-

[1] Scott M.P., Tamkun J.W., Hartzell G.W. III *Biochim. Biophys. Acta* 989:25-48(1989).

[2] Gehring W.J. *Science* 236:1245-1252(1987).

298. Isocitrate lyase signature (ICL)

Isocitrate lyase (EC 4.1.3.1) [1,2] is an enzyme that catalyzes the conversion of isocitrate to succinate and glyoxylate. This is the first step in the glyoxylate bypass, an alternative to the tricarboxylic acid cycle in bacteria, fungi and plants. A cysteine, a histidine and a glutamate or aspartate have been found to be important for the enzyme's catalytic activity. Only one cysteine residue is conserved between the sequences of the fungal, plant and bacterial enzymes; it is located in the middle of a conserved hexapeptide that can be used as a signature pattern for this type of enzyme.

10 Consensus pattern: K-[KR]-C-G-H-[LMO] [C is a putative active site residue]-

[1] Beeching J.R. Protein Seq. Data Anal. 2:463-466(1989).

[2] Atomi H., Ueda M., Hikida M., Hishida T., Teranishi Y., Tanaka A. J. Biochem. 107:262-266(1990).

299. Initiation factor 2 subunit

This family includes initiation factor 2B alpha, beta and delta subunits from eukaryotes, related proteins from archaeobacteria and IF-2 from prokaryotes. Initiation factor 2 binds to Met-tRNA, GTP and the small ribosomal subunit.

[1] Kyrpides NC, Woese CR, Proc Natl Acad Sci U S A 1998;95:3726-3730.

300. Initiation factor 3 signature

Initiation factor 3 (IF-3) (gene infC) [1] is one of the three factors required for the initiation of protein biosynthesis in bacteria. IF-3 is thought to function as a fidelity factor during the assembly of the ternary initiation complex which consist of the 30S ribosomal subunit, the initiator tRNA and the messenger RNA. IF-3 binds to the 30S ribosomal subunit; it is a basic protein of 141 to 212 residues. The chloroplast initiation factor IF-3(chl) is a protein that enhances the poly(A,U,G)-dependent binding of the initiator tRNA to chloroplast ribosomal 30S subunits. In its mature form it is a protein of about 400 residues whose central section is evolutionary related to the sequence of bacterial IF-3 [2]. As a signature pattern a highly conserved region was selected located in the central section of bacterial IF-3 and of IF-3(chl).

Consensus pattern: [KR]-[LIVM](2)-[DN]-[FY]-[GSN]-[KR]-[LIVMFYS]-x-[FY]-
[DEQTH]-x(2)-[KRO]-

- 5 [1] Liveris D., Schwartz J.J., Geertman R., Schwartz I. FEMS Microbiol. Lett. 112:211-216(1993).
[2] Lin Q., Ma L., Burkhart W., Spremulli L.L. J. Biol. Chem. 269:9436-9444(1994).

10 301. Imidazoleglycerol-phosphate dehydratase signatures (IGPD)

Imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19) is the enzyme that catalyzes the seventh step in the biosynthesis of histidine in bacteria, fungi and plants. In most organisms it is a monofunctional protein of about 22 to 29 Kd. In some bacteria such as *Escherichia coli* it is the C-terminal domain of a bifunctional protein that include a histidinol-phosphatase domain [1]. Two signature patterns were developed that each include two consecutive histidine residues.

Consensus pattern: [LIVMY]-[DE]-x-H-H-x(2)-E-x(2)-[GCA]-[LIVM]-[STAC]-[LIVM]-
Consensus pattern: G-x-[DN]-x-H-H-x(2)-E-[STAGC]-x-[FY]-K -

- 20 [1] Carlomagno M.S., Chiariotti L., Alifano P., Nappo A.G., Bruni C.B. J. Mol. Biol. 203:585-606(1988).

25 302. Indole-3-glycerol phosphate synthase signature (IGPS)

Indole-3-glycerol phosphate synthase (EC 4.1.1.48) (IGPS) catalyzes the fourth step in the biosynthesis of tryptophan: the ring closure of 1-(2-carboxy-phenylamino)-1-deoxyribulose into indol-3-glycerol-phosphate. In some bacteria, IGPS is a single chain enzyme. In others - such as *Escherichia coli* - it is the N-terminal domain of a bifunctional enzyme that also catalyzes N-(5'-phosphoribosyl)anthranilate isomerase (PRAI) activity, the third step of tryptophan biosynthesis. In fungi, IGPS is the central domain of a trifunctional enzyme that also contains a PRAI C-terminal domain and a glutamine amidotransferase N-terminal domain. The N-terminal section of IGPS contains a highly conserved region which X-ray

crystallography studies [1] have shown to be part of the active site cavity. This region was used as a signature pattern for IGPS.

Consensus pattern: [LIVMFY]-[LIVMC]-x-E-[LIVMFYC]-K-[KRSP]-[STAK]-S-P-[ST]-
5 x(3)-[LIVMFYST]-

[1] Wilmanns M., Priestle J.P., Niermann T., Jansonius J.N. J. Mol. Biol. 223:477-
507(1992).

10 303. (IL2) Interleukin 2. 31 members

304. (ILVD EDD) Dihydroxy-acid and 6-phosphogluconate dehydratases. Two dehydratases
15 have been shown [1] to be evolutionary related: - Dihydroxy-acid dehydratase (EC 4.2.1.9)
(gene *ilvD* or *ILV3*) which catalyzes the fourth step in the biosynthesis of isoleucine and
valine, the dehydration of 2,3-dihydroxy-isovaleric acid into alpha-ketoisovaleric acid. - 6-
phosphogluconate dehydratase (EC 4.2.1.12) (gene *edd*) which catalyzes the first step in the
Entner-Doudoroff pathway, the dehydration of 6-phospho- D-gluconate into 6-phospho-2-
20 dehydro-3-deoxy-D-gluconate. - Escherichia coli hypothetical protein *yjhG*. Both enzymes
are proteins of about 600 amino acid residues. Two highly conserved regions have been
developed as signature patterns. The first pattern is located in the N-terminal part and
contains a cysteine that could be involved in the binding of a 2Fe-2S iron-sulfur cluster [2].
The second pattern is located in the C-terminal half.

25 Consensus pattern: C-D-K-x(2)-P-[GA]-x(3)-[GA] [The C could be a 2Fe-2S ligand]
Consensus pattern: [SA]-L-[LIVM]-T-D-[GA]-R-[LIVMF]-S-[GA]-[GAV]-[ST]-

[1] Egan S.E., Fliege R., Tong S., Shibata A., Wolf R.E. Jr., Conway T. J. Bacteriol.
30 174:4638-4646(1992). [2] Velasco J.A., Cansado J., Pena M.C., Kawakami T., Laborda J.,
Notario V. Gene 137:179-185(1993).

305. IMP dehydrogenase / GMP reductase signature

IMP dehydrogenase (EC 1.1.1.205) (IMPDH) catalyzes the rate-limiting reaction of de novo GTP biosynthesis, the NAD-dependent reduction of IMP into XMP [1]. Inhibition of IMP dehydrogenase activity results in the cessation of DNA synthesis. As IMP dehydrogenase is associated with cell proliferation, it is a possible target for cancer chemotherapy. Mammalian and bacterial IMPDHs are tetramers of identical chains. There are two IMP dehydrogenase isozymes in humans [2]. GMP reductase (EC 1.6.6.8) catalyzes the irreversible and NADPH-dependent reductive deamination of GMP into IMP [3]. It converts nucleobase, nucleoside and nucleotide derivatives of G to A nucleotides, and maintains intracellular balance of A and G nucleotides. IMP dehydrogenase and GMP reductase share many regions of sequence similarity. One of these regions is centered on a cysteine residue thought [3] to be involved in binding IMP. This region was used as a signature pattern.

Consensus pattern: [LIVM]-[RK]-[LIVM]-G-[LIVM]-G-x-G-S-[LIVM]-C-x-T [C is the putative IMP-binding residue]-

[1] Collart F.R., Huberman E. J. Biol. Chem. 263:15769-15772(1988).

[2] Natsumeda Y., Ohno S., Kawasaki H., Konno Y., Weber G., Suzuki K. J. Biol. Chem. 265:5292-5295(1990).

[3] Andrews S.C., Guest J.R. Biochem. J. 255:35-43(1988).

306. (IPPC) Inositol polyphosphate phosphatase family, catalytic domain

[1] York JD, Ponder JW, Chen ZW, Mathews FS, Majerus PW;

Biochemistry 1994;33:13164-13171. [2] Jefferson AB, Auethavekiat V, Pot DA, Williams LT, Majerus PW; J Biol Chem 1997;272:5983-5988. [3] Zhang X, Jefferson AB, Auethavekiat V, Majerus PW; Proc Natl Acad Sci U S A 1995;92:4853-4856. [4] York JD, Majerus PW. Proc Natl Acad Sci U S A 1990;87:9548-9552. [5] Neuwald AF, York JD, Majerus PW;

FEBS Lett 1991;294:16-18.

307. IQ calmodulin-binding motif

[1] Xie X, Harrison DH, Schlichting I, Sweet RM, Kalabokis VN,

Szent-Gyorgyi AG, Cohen C; Nature 1994;368:306-312.

[2] Rhoads AR, Friedberg F; FASEB J 1997;11:331-340.

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308. Inosine-uridine preferring nucleoside hydrolase family signature (IU nuc hydro)
Inosine-uridine preferring nucleoside hydrolase (EC 3.2.2.1) (IU-nucleosidehydrolase or
IUNH) is an enzyme first identified in protozoan [1] that catalyzes the hydrolysis of all of the
commonly occurring purine and pyrimidine nucleosides into ribose and the associated base,
10 but has a preference for inosine and uridine as substrates. This enzyme is important for these
parasitic organisms, which are deficient in de novo synthesis of purines, to salvage the host
purine nucleosides. IUNH from Crithidia fasciculata has been sequenced and characterized, it
is an homotetrameric enzyme of subunits of 34 Kd. An histidine has been shown to be
important for the catalytic mechanism, it acts a proton donor to activate the hypoxanthine
15 leaving group. IUNH is evolutionary related to a number of uncharacterized proteins from
various biological sources, notably: - Escherichia coli hypothetical protein yaaF. -
Escherichia coli hypothetical protein ybeK. - Escherichia coli hypothetical protein yeiK. -
Fission yeast hypothetical protein SpAC17G8.02. - Yeast hypothetical protein YDR400w. -
An hypothetical protein from the archaeobacteria Desulfurolobus ambivalens. As a signature
20 pattern for these proteins, a highly conserved region was selected located in the N-terminal
extremity. This region contains four conserved aspartates that have been shown [2] to be
located in the active site cavity.

Consensus pattern: D-x-D-[PT]-[GA]-x-D-D-[TAV]-[VI]-A -

25

[1] Gopaul D.N., Meyer S.L., Degano M., Sacchettini J.C., Schramm V.L. Biochemistry
35:5963-5970(1996).

[2] Degano M., Gopaul D.N., Scapin G., Schramm V.L., Sacchettini J.C. Biochemistry
35:5971-5981(1996).

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309. (Insulinase)

Insulinase family, zinc-binding region signature

(aka Peptidase_M16)

A number of proteases dependent on divalent cations for their activity have been shown [1,2] to belong to one family, on the basis of sequence similarity. These enzymes are listed below.

5

- Insulinase (EC 3.4.24.56) (also known as insulysin or insulin-degrading enzyme or IDE), a cytoplasmic enzyme which seems to be involved in the cellular processing of insulin, glucagon and other small polypeptides.

10 - Escherichia coli protease III (EC 3.4.24.55) (pitrylsin) (gene ptr), a periplasmic enzyme that degrades small peptides.

15 - Mitochondrial processing peptidase (EC 3.4.24.64) (MPP). This enzyme removes the transit peptide from the precursor form of proteins imported from the cytoplasm across the mitochondrial inner membrane. It is composed of two nonidentical homologous subunits termed alpha and beta. The beta subunit seems to be catalytically active while the alpha subunit has probably lost its activity.

- Nardilysin (EC 3.4.24.61) (N-arginine dibasic convertase or NRD convertase) this mammalian enzyme cleaves peptide substrates on the N-terminus of Arg residues in dibasic stretches.

20 - Klebsiella pneumoniae protein pqqF. This protein is required for the biosynthesis of the coenzyme pyrrolo-quinoline-quinone (PQQ). It is thought to be protease that cleaves peptide bonds in a small peptide (gene pqqA) thus providing the glutamate and tyrosine residues necessary for the synthesis of PQQ.

- Yeast protein AXL1, which is involved in axial budding [3].

- Eimeria bovis sporozoite developmental protein.

25 - Escherichia coli hypothetical protein yddC and H11368, the corresponding Haemophilus influenzae protein.

- Bacillus subtilis hypothetical protein ymxG.

- Caenorhabditis elegans hypothetical proteins C28F5.4 and F56D2.1.

30 It should be noted that in addition to the above enzymes, this family also includes the core proteins I and II of the mitochondrial bc1 complex (also called cytochrome c reductase or complex III), but the situation as to the activity or lack of activity of these subunits is quite complex:

- In mammals and yeast, core proteins I and II lack enzymatic activity.
- In *Neurospora crassa* and in potato protein I is equivalent to the beta subunit of MPP.
- In *Euglena gracilis*, core protein I seems to be active, while subunit II is inactive.

5 These proteins do not share many regions of sequence similarity; the most noticeable is in the N-terminal section. This region includes a conserved histidine followed, two residues later by a glutamate and another histidine. In ptilin, it has been shown [4] that this H-x-x-E-H motif is involved in enzyme activity; the two histidines bind zinc and the glutamate is necessary for catalytic activity. Non active members of this family have lost from one to three
10 of these active site residues. We developed a signature pattern that detect active members of this family as well as some inactive members.

Consensus pattern G-x(8,9)-G-x-[STA]-H-[LIVMFY]-[LIVMC]-[DERN]-[HRKL]-
[LMFAT]-x-[LFSTH]-x-[GSTAN]-[GST] [The two H are zinc ligands] [E is the active site
15 residue] Sequences known to belong to this class detected by the pattern ALL active members as well as all MPP alpha subunits and core II subunits. Does not detect inactive core I subunits.

Note: these proteins belong to family M16 in the classification of peptidases [5].

[1] Rawlings N.D., Barrett A.J. Biochem. J. 275:389-391(1991).

[2] Braun H.-P., Schmitz U.K. Trends Biochem. Sci. 20:171-175(1995).

25 [3] Becker A.B., Roth R.A. Proc. Natl. Acad. Sci. U.S.A. 89:3835-3839(1992).

[4] Fujita A., Oka C., Arikawa Y., Katagai T., Tonouchi A., Kuhara S., Misumi Y. Nature 372:567-570(1994).

[5] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

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310. Involucrin repeat

Eckert RL, Yaffe MB, Crish JF, Murthy S, Rorke EA, Welter JF, J Invest Dermatol 1993;100:613-617.

311. Isochorismatase family. This family are hydrolase enzymes.

- Romao MJ, Turk D, Gomis-Ruth FX, Huber R, Schumacher G, Mollering H, Russmann L, J
5 Mol Biol 1992;226:1111-1130.

312. Inositol monophosphatase family signatures (inositol_P)

- It has been shown [1] that several proteins share two sequence motifs. Two of these proteins
10 are enzymes of the inositol phosphate second messenger signaling pathway: - Vertebrate and
plants inositol monophosphatase (EC 3.1.3.25). - Vertebrate inositol polyphosphate 1-
phosphatase (EC 3.1.3.57). The function of the other proteins is not yet clear: - Bacterial
protein cysQ. CysQ could help to control the pool of PAPS (3'-phosphoadenosine 5'-
phosphosulfate), or be useful in sulfite synthesis. - Escherichia coli protein suhB. Mutations
15 in suhB results in the enhanced synthesis of heat shock sigma factor (htpR). - Neurospora
crassa protein Qa-X. Probably involved in quinate metabolism. - Emericella nidulans protein
qutG. Probably involved in quinate metabolism. - Yeast protein HAL2/MET22 [2] involved
in salt tolerance as well as methionine biosynthesis. - Yeast hypothetical protein
YHR046c. - Caenorhabditis elegans hypothetical protein F13G3.5. - A Rhizobium
20 leguminosarum hypothetical protein encoded upstream of the pss gene for exopolysaccharide
synthesis. - Methanococcus jannaschii hypothetical protein MJ0109. It is suggested [1] that
these proteins may act by enhancing the synthesis or degradation of phosphorylated
messenger molecules. From the X-ray structure of human inositol monophosphatase [3], it
seems that some of the conserved residues are involved in binding a metal ion and/or the
25 phosphate group of the substrate.

Consensus pattern: [FWV]-x(0,1)-[LIVM]-D-P-[LIVM]-D-[SG]-[ST]-x(2)-[FY]-x-
[HKRNSTY] [The first D and the T bind a metal ion]-

- Consensus pattern: [WV]-D-x-[AC]-[GSA]-[GSAPV]-x-[LIVACP]-[LIV]-[LIVAC]-x(3)-
30 [GH]-[GA]-

[1] Neuwald A.F., York J.D., Majerus P.W. FEBS Lett. 294:16-18(1991).

[2] Glaeser H.-U., Thomas D., Gaxiola R., Montrichard F., Surdin-Kerjan Y., Serrano R.
EMBO J. 12:3105-3110(1993).

[3] Bone R., Springer J.P., Atack J.R. Proc. Natl. Acad. Sci. U.S.A. 89:10031-10035(1992).

313. Ion transport protein

- 5 This family contains Sodium, Potassium, Calcium ion channel. This family is 6 transmembrane helices in which the last two helices flank a loop which determines ion selectivity. In some sub-families (e.g. Na channels) the domain is repeated four times, whereas in others (e.g. K channels) the protein forms as a tetramer in the membrane. A bacterial structure of the protein is known for the last two helices but is not the Pfam family
10 due to it lacking the first four helices

314. Isocitrate and isopropylmalate dehydrogenases signature (isodh)

- Isocitrate dehydrogenase (IDH) [1,2] is an important enzyme of carbohydrate metabolism
15 which catalyzes the oxidative decarboxylation of isocitrate into alpha-ketoglutarate. IDH is either dependent on NAD⁺ (EC 1.1.1.41) or on NADP⁺ (EC 1.1.1.42). In eukaryotes there are at least three isozymes of IDH: two are located in the mitochondrial matrix (one NAD⁺-dependent, the other NADP⁺-dependent), while the third one (also NADP⁺-dependent) is cytoplasmic. In *Escherichia coli* the activity of a NADP⁺-dependent form of the enzyme is
20 controlled by the phosphorylation of a serine residue; the phosphorylated form of IDH is completely inactivated. 3-isopropylmalate dehydrogenase (EC 1.1.1.85) (IMDH) [3,4] catalyzes the third step in the biosynthesis of leucine in bacteria and fungi, the oxidative decarboxylation of 3-isopropylmalate into 2-oxo-4-methylvalerate. Tartrate dehydrogenase (EC 1.1.1.93) [5] catalyzes the reduction of tartrate to oxalglycolate. These enzymes are
25 evolutionary related [1,3,4,5]. The best conserved region of these enzymes is a glycine-rich stretch of residues located in the C-terminal section. This region was used as a signature pattern.

- Consensus pattern: [NS]-[LIMYT]-[FYDN]-G-[DNT]-[IMVY]-x-[STGDN]-[DN]-x(2)-
30 [SGAP]-x(3,4)-G-[STG]-[LIVMPA]-G-[LIVMF]-

[1] Hurley J.H., Thorsness P.E., Ramalingam V., Helmers N.H., Koshland D.E. Jr., Stroud R.M. Proc. Natl. Acad. Sci. U.S.A. 86:8635-8639(1989).

[2] Cupp J.R., McAlister-Henn L. J. Biol. Chem. 266:22199-22205(1991).

[3] Imada K., Sato M., Tanaka N., Katsube Y., Matsuura Y., Oshima T. J. Mol. Biol. 222:725-738(1991).

[4] Zhang T., Koshland D.E. Jr. Protein Sci. 4:84-92(1995).

[5] Tipton P.A., Beecher B.S. Arch. Biochem. Biophys. 313:15-21(1994).

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315. Jacalin-like lectin domain.

Proteins containing this domain are lectins. It is found in

10 1 to 6 copies in these proteins. The domain is also found in the animal prostatic spermine-binding protein (Swiss:PI5501).

[1] Sankaranarayanan R, Sekar K, Banerjee R, Sharma V, Surolia A, Vijayan M; Nat Struct Biol 1996;3:596-603.

15

316. KH domain

KH motifs probably bind RNA directly. Auto antibodies to Nova, a KH domain protein, cause paraneoplastic opsoclonus ataxia.

20 [1] Burd CG, Dreyfuss G, Science 1994;265:615-621.

[2] Musco G, Stier G, Joseph C, Castiglione Morelli MA, Nilges M, Gibson TJ, Pastore A, Cell 1996;85:237-245.

25 317. Kelch motif

The kelch motif was initially discovered in Kelch (Swiss:Q04652). In this protein there are six copies of the motif. It has been shown that Swiss:Q04652 is related to Galactose Oxidase [1] for which a structure has been solved [2]. The kelch motif forms a beta sheet. Several of these sheets associate to form a beta propeller structure as found in neur,

30

[1] Bork P, Doolittle RF, J Mol Biol 1994;236:1277-1282. [2] Ito N, Phillips SE, Stevens C, Ogel ZB, McPherson MJ, Keen, JN, Yadav KD, Knowles PF, Nature 1991;350:87-90.

318. Soybean trypsin inhibitor (Kunitz) protease inhibitors family signature

- The soybean trypsin inhibitor (Kunitz) family [1] is one of the numerous families of proteinase inhibitors. It comprise plant proteins which have inhibitory activity against serine proteinases from the trypsin and subtilisin families, thiol proteinases and aspartic proteinases as well as some proteins that are probably involved in seed storage. This family is currently known to group the following proteins: - Trypsin inhibitors A, B, C, KTI1, and KTI2 from soybean. - Trypsin inhibitor DE3 from coral beans (*Erythrina* sp.). - Trypsin inhibitor DES from sandal bead tree. - Trypsin inhibitors 1A (WTI-1A), 1B (WTI-1B), and 2 (WTI-2) from goa bean. - Trypsin inhibitor from *Acacia confusa*. - Trypsin inhibitor from silk tree. - Chymotrypsin inhibitor 3 (WCI-3) from goa bean. - Cathepsin D inhibitors PDI and NDI from potato [2], which inhibit both cathepsin D (aspartic proteinase) and trypsin. - Alpha-amylase/subtilisin inhibitors from barley and wheat. - Albumin-1 (WBA-1) from goa bean seeds [3]. - Miraculin from *Richadella dulcifica* [4], a sweet taste protein. - Sporamin from sweet potato [5], the major tuberous root protein. - Thiol proteinase inhibitor PCPI 8.3 (P340) from potato tuber [6]. - Wound responsive protein gwin3 from poplar tree [7]. - 21 Kd seed protein from cocoa [8]. All these proteins contain from 170 to 200 amino acid residues and one or two intrachain disulfide bonds. The best conserved region is found in their N-terminal section and is used as a signature pattern
- 20 Consensus pattern: [LIVM]-x-D-x-[EDNTY]-[DG]-[RKHDENQ]-x-[LIVM]-x(5)-Y-x-[LIVM]-

[1] Laskowski M., Kato I. Annu. Rev. Biochem. 49:593-626(1980).

- [2] Ritonja A., Krizaj I., Mesko P., Kopitar M., Lucovnik P., Strukelj B., Pungercar J., Buttle D.J., Barrett A.J., Turk V. FEBS Lett. 267:13-15(1990).

[3] Kortt A.A., Strike P.M., de Jersey J. Eur. J. Biochem. 181:403-408(1989).

[4] Theerasilp S., Hitotsuya H., Nakajo S., Nakaja K., Nakamura Y., Kurihara Y. J. Biol. Chem. 264:6655-6659(1989).

[5] Hattori T., Yoshida N., Nakamura K. Plant Mol. Biol. 13:563-572(1989).

- [6] Krizaj I., Drobnic-Kosorok M., Brzin J., Jerala R., Turk V. FEBS Lett. 333:15-20(1993).

[7] Bradshaw H.D., Hollick J.B., Parsons T.J., Clarke H.R.G., Gordon M.P. Plant Mol. Biol. 14:51-59(1989).

[8] Tai H., McHenry L., Fritz P.J., Furtek D.B. Plant Mol. Biol. 16:913-915(1991).

319. Beta-ketoacyl synthases active site

- Beta-ketoacyl-ACP synthase (KAS) [1] is the enzyme that catalyzes the condensation of malonyl-ACP with the growing fatty acid chain. It is found as a component of the following
- 5 enzymatic systems: - Fatty acid synthetase (FAS), which catalyzes the formation of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH. Bacterial and plant chloroplast FAS are composed of eight separate subunits which correspond to different enzymatic activities; beta-ketoacyl synthase is one of these polypeptides. Fungal FAS consists of two multifunctional proteins, FAS1 and FAS2; the beta-ketoacyl synthase domain
 - 10 is located in the C-terminal section of FAS2. Vertebrate FAS consists of a single multifunctional chain; the beta-ketoacyl synthase domain is located in the N-terminal section [2]. - The multifunctional 6-methylsalicylic acid synthase (MSAS) from *Penicillium patulum* [3]. This is a multifunctional enzyme involved in the biosynthesis of a polyketide antibiotic and which has a KAS domain in its N-terminal section. - Polyketide antibiotic synthase
 - 15 enzyme systems. Polyketides are secondary metabolites produced by microorganisms and plants from simple fatty acids. KAS is one of the components involved in the biosynthesis of the *Streptomyces* polyketide antibiotics granatacin [4], tetracenomyacin C [5] and erythromycin. - *Emericella nidulans* multifunctional protein Wa. Wa is involved in the biosynthesis of conidial green pigment. Wa is protein of 216 Kd that contains a KAS domain.
 - 20 - Rhizobiurn nodulation protein node, which probably acts as a beta-ketoacyl synthase in the synthesis of the nodulation Nod factor fatty acyl chain. - Yeast mitochondrial protein CEM1. The condensation reaction is a two step process: the acyl component of an activated acyl primer is transferred to a cysteine residue of the enzyme and is then condensed with an activated malonyl donor with the concomitant release of carbon dioxide. The sequence
 - 25 around the active site cysteine is well conserved and can be used as a signature pattern.

Consensus pattern: G-x(4)-[LIVMFAP]-x(2)-[AGC]-C-[STA](2)-[STAG]-x(3)-[LIVMF] [C is the active site residue]

- 30 [1] Kauppinen S., Siggaard-Andersen M., von Wettstein-Knowles P. *Carlsberg Res. Commun.* 53:357-370(1988).
- [2] Witkowski A., Rangan V.S., Randhawa Z.I., Amy C.M., Smith S. *Eur. J. Biochem.* 198:571-579(1991).

- [3] Beck J., Ripka S., Siegner A., Schiltz E., Schweizer E. *Eur. J. Biochem.* 192:487-498(1990).
- [4] Bibb M.J., Biro S., Motamedi H., Collins J.F., Hutchinson C.R. *EMBO J.* 8:2727-2736(1989).
- 5 [5] Sherman D.H., Malpartida F., Bibb M.J., Kieser H.M., Bibb M.J., Hopwood D.A. *EMBO J.* 8:2717-2725(1989).

320. Kinesin motor domain signature and profile

- 10 Kinesin [1,2,3] is a microtubule-associated force-producing protein that may play a role in organelle transport. Kinesin is an oligomeric complex composed of two heavy chains and two light chains. The kinesin motor activity is directed toward the microtubule's plus end. The heavy chain is composed of three structural domains: a large globular N-terminal domain which is responsible for the motor activity of kinesin (it is known to hydrolyze ATP, to bind and move on microtubules), a central alpha-helical coiled coil domain that mediates the heavy chain dimerization; and a small globular C-terminal domain which interacts with other proteins (such as the kinesin light chains), vesicles and membranous organelles. A number of proteins have been recently found that contain a domain similar to that of the kinesin 'motor' domain [1,4,E1]: - *Drosophila* claret segregational protein (ncd). Ncd is required for normal chromosomal segregation in meiosis, in females, and in early mitotic divisions of the embryo. The ncd motor activity is directed toward the microtubule's minus end. - *Drosophila* kinesin-like protein (nod). Nod is required for the distributive chromosome segregation of nonexchange chromosomes during meiosis. - Human CENP-E [4]. CENP-E is a protein that associates with kinetochores during chromosome congression, relocates to the spindle midzone at anaphase, and is quantitatively discarded at the end of the cell division. CENP-E is probably an important motor molecule in chromosome movement and/or spindle elongation. - Human mitotic kinesin-like protein-1 (MKLP-1), a motor protein whose activity is directed toward the microtubule's plus end. - Yeast KAR3 protein, which is essential for yeast nuclear fusion during mating. KAR3 may mediate microtubule sliding during nuclear fusion and possibly mitosis. - Yeast CIN8 and KIP1 proteins which are required for the assembly of the mitotic spindle. Both proteins seem to interact with spindle microtubules to produce an outwardly directed force acting upon the poles. - Fission yeast cut7 protein, which is essential for spindle body duplication during mitotic division. - *Emericella nidulans* bimC, which plays an important role in nuclear division. - *Emericella nidulans* klpA. -

Caenorhabditis elegans unc-104, which may be required for the transport of substances needed for neuronal cell differentiation. - Caenorhabditis elegans osm-3. - Xenopus Eg5, which may be involved in mitosis. - Arabidopsis thaliana KatA, KatB and katC. - Chlamydomonas reinhardtii FLA10/KHP1 and KLP1. Both proteins seem to play a role in the rotation or twisting of the microtubules of the flagella. - Caenorhabditis elegans hypothetical protein T09A5.2. The kinesin motor domain is located in the N-terminal part of most of the above proteins, with the exception of KAR3, klpA, and ncd where it is located in the C-terminal section. The kinesin motor domain contains about 330 amino acids. An ATP-binding motif of type A is found near position 80 to 90, the C-terminal half of the domain is involved in microtubule-binding. The signature pattern for that domain is derived from a conserved decapeptide inside the microtubule-binding part.

Consensus pattern: [GSA]-[KRHPSTQVM]-[LIVMF]-x-[LIVMF]-[IVC]-D-L-[AH]-G-[SAN]-E

- [1] Bloom G.S., Endow S.A. Protein Prof. 2:1109-1171(1995).
- [2] Vallee R.B., Shpetner H.S. Annu. Rev. Biochem. 59:909-932(1990).
- [3] Brady S.T. Trends Cell Biol. 5:159-164(1995).
- [4] Endow S.A. Trends Biochem. Sci. 16:221-225(1991).[E1]

321. Ribosomal protein L15 signature

Ribosomal protein L15 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L15 is known to bind the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L15. - Plant chloroplast L15 (nuclear-encoded). - Archaeobacterial L15. - Vertebrate L27a. - Tetrahymena thermophila L29. - Fungi L27a (L29, CRP-1, CYH2). L15 is a protein of 144 to 154 amino-acid residues. As a signature pattern, a conserved region was selected in the C-terminal section of these proteins.

Consensus pattern: K-[LIVM](2)-[GASL]-x-[GT]-x-[LIVMA]-x(2,5)-[LIVM]-x-[LIVMF]-x(3,4)-[LIVMFCA]-[ST]-x(2)-A-x(3)-[LIVM]-x(3)-G

- [1] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

322. LBP / BPI / CETP family signature

The following mammalian lipid-binding serum glycoproteins belong to the same family

- 5 [1,2,3]: - Lipopolysaccharide-binding protein (LBP). LBP binds to the lipid A moiety of bacterial lipopolysaccharides (LPS), a glycolipid present in the outer membrane of all Gram-negative bacteria. The LBP/LPS complex seems to interact with the CD14 receptor and may be responsible for the secretion of alpha-TNF. - Bactericidal permeability-increasing protein (BPI). Like LBP, BPI binds LPS and has a cytotoxic activity on Gram-negative bacteria. -
- 10 Cholesteryl ester transfer protein (CETP). CETP is involved in the transfer of insoluble cholesteryl esters in reverse cholesterol transport. - Phospholipid transfer protein (PLTP). May play a key role in extracellular phospholipid transport and modulation of HDL particles. These proteins are structurally related and share many regions of sequence similarities. As a signature pattern one of these regions was selected, which is located in the N-terminal section
- 15 of these proteins; a region which could be involved in the binding to the lipids [2].

Consensus pattern: [PA]-[GA]-[LIVMC]-x(2)-R-[IV]-[ST]-x(3)-L-x(5)-[EQ]-x(4)-[LIVM]-[EQK]-x(8)-P

- 20 [1] Schumann R.R., Leong S.R., Flaggs G.W., Gray P.W., Wright S.D., Mathison J.C., Tobias P.S., Ulevitch R.J. Science 249:1429-1431(1990).
- [2] Gray P.W., Flaggs G., Leong S.R., Gumina R.J., Weiss J., Ooi C.E., Elsbach P. J. Biol. Chem. 264:9505-9509(1989).
- [3] Day J.R., Albers J.J., Lofton-Day C.E., Gilbert T.L., Ching A.F.T., Grant F.J., O'Hara
- 25 P.J., Marcovina S.M., Adolphson J.L. J. Biol. Chem. 269:9388-9391(1994).

323. LIM domain signature and profile

Recently [1,2] a number of proteins have been found to contain a conserved cysteine-rich domain of about 60 amino-acid residues. These proteins are: - *Caenorhabditis elegans* mec-3; a protein required for the differentiation of the set of six touch receptor neurons in this nematode. - *Caenorhabditis elegans* lin-11; a protein required for the asymmetric division of vulval blast cells. - Vertebrate insulin gene enhancer binding protein isl-1. Isl-1 binds to one of the two cis-acting protein-binding domains of the insulin gene. - Vertebrate homeobox

- proteins *lim-1*, *lim-2* (*lim-5*) and *lim-3*. - Vertebrate *lmx-1*, which acts as a transcriptional activator by binding to the FLAT element; a beta-cell-specific transcriptional enhancer found in the insulin gene. - Mammalian *LH-2*, a transcriptional regulatory protein involved in the control of cell differentiation in developing lymphoid and neural cell types. - *Drosophila*
- 5 protein *apterous*, required for the normal development of the wing and halter imaginal discs. - Vertebrate protein kinases *LIMK-1* and *LIMK-2*. - Mammalian rhombotins. Rhombotin 1 (*RBTN1* or *TTG-1*) and rhombotin-2 (*RBTN2* or *TTG-2*) are proteins of about 160 amino acids whose genes are disrupted by chromosomal translocations in T-cell leukemia. - Mammalian and avian cysteine-rich protein (*CRP*), a 192 amino-acid protein of unknown
 - 10 function. Seems to interact with zyxin. - Mammalian cysteine-rich intestinal protein (*CRIP*), a small protein which seems to have a role in zinc absorption and may function as an intracellular zinc transport protein. - Vertebrate *paxillin*, a cytoskeletal focal adhesion protein. - Mouse *testin*. Mouse *testin* should not be confused with rat *testin* which is a thiol protease homolog. - Sunflower pollen specific protein *SF3*. - Chicken *zyxin*. *Zyxin* is a low-abundance
 - 15 adhesion plaque protein which has been shown to interact with *CRP*. - Yeast protein *LRG1* which is involved in sporulation [4]. - Yeast rho-type GTPase activating protein *RGA1/DBM1*. - *Caenorhabditis elegans* homeobox protein *ceh-14*. - *Caenorhabditis elegans* homeobox protein *unc-97*. - Yeast hypothetical protein *YKR090w*. - *Caenorhabditis elegans* hypothetical proteins *C28H8.6*. These proteins generally have two tandem copies of a domain,
 - 20 called *LIM* (for *Lin-11 Isl-1 Mec-3*) in their N-terminal section. *Zyxin* and *paxillin* are exceptions in that they contain respectively three and four *LIM* domains at their C-terminal extremity. In *apterous*, *isl-1*, *LH-2*, *lin-11*, *lim-1* to *lim-3*, *lmx-1* and *ceh-14* and *mec-3* there is a homeobox domain some 50 to 95 amino acids after the *LIM* domains. In the *LIM* domain, there are seven conserved cysteine residues and a histidine. The arrangement
 - 25 followed by these conserved residues is C-x(2)-C-x(16,23)-H-x(2)-[CH]-x(2)-C-x(2)-C-x(16,21)-C-x(2,3)-[CHD]. The *LIM* domain binds two zinc ions [5]. *LIM* does not bind DNA, rather it seems to act as an interface for protein-protein interaction. A pattern was developed that spans the first half of the *LIM* domain.
 - 30 Consensus pattern: C-x(2)-C-x(15,21)-[FYWH]-H-x(2)-[CH]-x(2)-C-x(2)-C-x(3)-[LIVMF]
[The 5 C's and the H bind zinc]

[1] Freyd G., Kim S.K., Horvitz H.R. Nature 344:876-879(1990).

[2] Baltz R., Evrard J.-L., Domon C., Steinmetz A. Plant Cell 4:1465-1466(1992).

- [3] Sanchez-Garcia I., Rabbitts T.H. Trends Genet. 10:315-320(1994).
 [4] Mueller A., Xu G., Wells R., Hollenberg C.P., Piepersberg W. Nucleic Acids Res. 22:3151-3154(1994).
 [5] Michelsen J.W., Schmeichel K.L., Beckerle M.C., Winge D.R. Proc. Natl. Acad. Sci. U.S.A. 90:4404-4408(1993).

324. (LRR) Leucine Rich Repeat

CAUTION: This Pfam may not find all Leucine Rich Repeats in a protein. Leucine Rich Repeats are short sequence motifs present in a number of proteins with diverse functions and cellular locations. These repeats are usually involved in protein-protein interactions. Each Leucine Rich Repeat is composed of a beta-alpha unit. These units form elongated non-globular structures. Leucine Rich Repeats are often flanked by cysteine rich domains. Number of members: 3017

- [1] The leucine-rich repeat: a versatile binding motif. Kobe B, Deisenhofer J; Trends Biochem Sci 1994;19:415-421. [2] Crystal structure of porcine ribonuclease inhibitor, a protein with leucine-rich repeats. Kobe B, Deisenhofer J; Nature 1993;366:751-756.

325. Plant lipid transfer protein family signature (LTP)

Plant cells contain proteins, called lipid transfer proteins (LTP) [1,2,3], which are able to facilitate the transfer of phospholipids and other lipids across membranes. These proteins, whose subcellular location is not yet known, could play a major role in membrane biogenesis by conveying phospholipids such as waxes or cutin from their site of biosynthesis to membranes unable to form these lipids. Plant LTP's are proteins of about 9 Kd (90 amino acids) which contain eight conserved cysteine residues all involved in disulfide bridges, as shown in the following schematic representation.

```

+-----+ | +-----+ ||| | *****
xCxxxxCxxxxxCxxxxxCxCCCCCCCCCxxxxxCxx ||| +-----|-----+ | +---
-----+

```

'C': conserved cysteine involved in a disulfide bond.

'*': position of the pattern.

[2] Holness C.L., da Silva R.P., Fawcett J., Gordon S., Simmons D.L. J. Biol. Chem. 268:9661-9666(1993).

5 327. Lipolytic enzymes "G-D-S-L" family, serine active site

Recently [1], a family of lipolytic enzymes has been characterized. This family currently consist of the following proteins:

- *Aeromonas hydrophila* lipase/phosphatidylcholine-sterol acyltransferase.

- *Xenorhabdus luminescens* lipase 1.

10 - *Vibrio mimicus* arylesterase.

- *Escherichia coli* acyl-coA thioesterase I (gene tesA).

- *Vibrio parahaemolyticus* thermolabile hemolysin/atypical phospholipase.

- Rabbit phospholipase AdRab-B, an intestinal brush border protein with esterase and phospholipase A/lysophospholipase activity that could be involved in the uptake of dietary
15 lipids. AdRab-B contains four repeats of about 320 amino acids.

- *Arabidopsis thaliana* and *Brassica napus* anther-specific proline-rich protein APG.

- A *Pseudomonas putida* hypothetical protein in trpE-trpG intergenic region. A serine has been identified a part of the active site in the *Aeromonas*, *Vibrio mimicus* and *Escherichia coli* enzymes. It is located in a conserved sequence motif that can be used as a signature
20 pattern for these proteins.

-Consensus pattern: [LIVMFYAG](4)-G-D-S-[LIVM]-x(1,2)-[TAG]-G
[S is the active site residue]

25

328. (Lipoprotein 4) Prokaryotic membrane lipoprotein lipid attachment site

In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which is cleaved by a specific lipoprotein signal peptidase (signalpeptidase II). The peptidase recognizes a conserved sequence and cuts upstream of a cysteine residue to which a glyceride-

30 fatty acid lipid is attached [1]. Some of the proteins known to undergo such processing currently include (for recent listings see [1,2,3]): - Major outer membrane lipoprotein (murein-lipoproteins) (gene lpp). - *Escherichia coli* lipoprotein-28 (gene nlpA). - *Escherichia coli* lipoprotein-34 (gene nlpB). - *Escherichia coli* lipoprotein nlpC. - *Escherichia coli* lipoprotein nlpD. - *Escherichia coli* osmotically inducible lipoprotein B (gene osmB). -

Escherichia coli osmotically inducible lipoprotein E (gene *osmE*). - *Escherichia coli* peptidoglycan-associated lipoprotein (gene *pal*). - *Escherichia coli* rare lipoproteins A and B (genes *rplA* and *rplB*). - *Escherichia coli* copper homeostasis protein *cutF* (or *nlpE*). - *Escherichia coli* plasmids *traT* proteins. - *Escherichia coli* Col plasmids lysis proteins. - A number of *Bacillus* beta-lactamases. - *Bacillus subtilis* periplasmic oligopeptide-binding protein (gene *oppA*). - *Borrelia burgdorferi* outer surface proteins A and B (genes *ospA* and *ospB*). - *Borrelia hermsii* variable major protein 21 (gene *vmp21*) and 7 (gene *vmp7*). - *Chlamydia trachomatis* outer membrane protein 3 (gene *omp3*). - *Fibrobacter succinogenes* endoglucanase *cel-3*. - *Haemophilus influenzae* proteins *Pal* and *Pcp*. - *Klebsiella pullulunase* (gene *pulA*). - *Klebsiella pullulunase* secretion protein *pulS*. - *Mycoplasma hyorhinis* protein *p37*. - *Mycoplasma hyorhinis* variant surface antigens A, B, and C (genes *vlpABC*). - *Neisseria* outer membrane protein H.8. - *Pseudomonas aeruginosa* lipopeptide (gene *lppL*). - *Pseudomonas solanacearum* endoglucanase *ecl*. - *Rhodospseudomonas viridis* reaction center cytochrome subunit (gene *cytC*). - Rickettsia 17 Kd antigen. - *Shigella flexneri* invasion plasmid proteins *mxjI* and *mxjM*. - *Streptococcus pneumoniae* oligopeptide transport protein A (gene *amiA*). - *Treponema pallidum* 34 Kd antigen. - *Treponema pallidum* membrane protein A (gene *tmpA*). - *Vibrio harveyi* chitobiase (gene *chb*). - *Yersinia* virulence plasmid protein *yscJ*. - Halocyanin from *Natrobacterium pharaonis* [4], a membrane associated copper-binding protein. This is the first archaeobacterial protein known to be modified in such a fashion). From the precursor sequences of all these proteins, a consensus pattern and a set of rules to identify this type of post-translational modification was derived.

Consensus pattern: {DERK}{6}-[LIVMFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1) The cysteine must be between positions 15 and 35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the first seven positions of the sequence.

[1] Hayashi S., Wu H.C. J. Bioenerg. Biomembr. 22:451-471(1990).

[2] Klein P., Somorjai R.L., Lau P.C.K. Protein Eng. 2:15-20(1988).

[3] von Heijne G. Protein Eng. 2:531-534(1989).

[4] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

329. (Lipoprotein 5) Prokaryotic membrane lipoprotein lipid attachment site. In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which is cleaved by a specific lipoprotein signal peptidase (signal peptidase II). The peptidase recognizes a conserved sequence and cuts upstream of a cysteine residue to which a glyceride-fatty acid lipid is attached [1]. Some of the proteins known to undergo such processing currently include (for recent listings see [1,2,3]): - Major outer membrane lipoprotein (murein-lipoproteins) (gene lpp). - *Escherichia coli* lipoprotein-28 (gene nlpA). - *Escherichia coli* lipoprotein-34 (gene nlpB). - *Escherichia coli* lipoprotein nlpC. - *Escherichia coli* lipoprotein nlpD. - *Escherichia coli* osmotically inducible lipoprotein B (gene osmB). - *Escherichia coli* osmotically inducible lipoprotein E (gene osmE). - *Escherichia coli* peptidoglycan-associated lipoprotein (gene pal). - *Escherichia coli* rare lipoproteins A and B (genes rplA and rplB). - *Escherichia coli* copper homeostasis protein cutF (or nlpE). - *Escherichia coli* plasmids traT proteins. - *Escherichia coli* Col plasmids lysis proteins. - A number of *Bacillus* beta-lactamases. - *Bacillus subtilis* periplasmic oligopeptide-binding protein (gene oppA). - *Borrelia burgdorferi* outer surface proteins A and B (genes ospA and ospB). - *Borrelia hermsii* variable major protein 21 (gene vmp21) and 7 (gene vmp7). - *Chlamydia trachomatis* outer membrane protein 3 (gene omp3). - *Fibrobacter succinogenes* endoglucanase cel-3. - *Haemophilus influenzae* proteins Pal and Pcp. - *Klebsiella* pullulanase (gene pulA). - *Klebsiella* pullulanase secretion protein pulS. - *Mycoplasma hyorhinis* protein p37. - *Mycoplasma hyorhinis* variant surface antigens A, B, and C (genes vlp ABC). - *Neisseria* outer membrane protein H.S. - *Pseudomonas aeruginosa* lipopeptide (gene lppL). - *Pseudomonas solanacearum* endoglucanase egl. - *Rhodospseudomonas viridis* reaction center cytochrome subunit (gene cytC). - Rickettsia 17 Kd antigen. - *Shigella flexneri* invasion plasmid proteins mxjI and mxjM. - *Streptococcus pneumoniae* oligopeptide transport protein A (gene amiA). - *Treponema pallidum* 34 Kd antigen. - *Treponema pallidum* membrane protein A (gene tmpA). - *Vibrio harveyi* chitobiase (gene chb). - *Yersinia* virulence plasmid protein yscJ. - Halocyanin from *Natrobacterium pharaonis* [4], a membrane associated copper-binding protein. This is the first archaeobacterial protein known to be modified in such a fashion). From the precursor sequences of all these proteins, a consensus pattern and a set of rules to identify this type of post-translational modification have been developed.

Consensus pattern: {DERK}{6}-[LIVMFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1) The cysteine must be between positions 15 and

35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the first seven positions of the sequence.

[1] Hayashi S., Wu H.C. J. Bioenerg. Biomembr. 22:451-471(1990).[2] Klein P., Somorjai R.L., Lau P.C.K. Protein Eng. 2:15-20(1988).[3] von Heijne G. Protein Eng. 2:531-534(1989).[4] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterheld D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

- 10 330. (Lum binding) Riboflavin synthase alpha chain family Lum-binding site signature
The following proteins have been shown [1,2] to be structurally and evolutionary related: -
Riboflavin synthase alpha chain (RS-alpha) (gene ribC in Escherichia coli, ribB in Bacillus subtilis and Photobacterium leiognathi, RIB5 in yeast). This enzyme synthesizes riboflavin from two moles of 6,7- dimethyl-8-(1'-D-ribityl)lumazine (Lum), a pteridine-derivative. -
15 Photobacterium phosphoreum lumazine protein (LumP) (gene luxL). LumP is a protein that modulates the color of the bioluminescence emission of bacterial luciferase. In the presence of LumP, light emission is shifted to higher energy values (shorter wavelength). LumP binds non-covalently to 6,7-dimethyl-8-(1'-D-ribityl) lumazine. - Vibrio fischeri yellow fluorescent protein (YFP) (gene luxY). Like LumP, YFP modulates light emission but towards a longer
20 wavelength. YFP binds non-covalently to FMN. These proteins seem to have evolved from the duplication of a domain of about 100 residues. In its C-terminal section, this domain contains a conserved motif [KR]-V-N-[LI]-E which has been proposed to be the binding site for Lum.RS-alpha which binds two molecules of Lum has two perfect copies of this motif, while LumP which binds one molecule of Lum, has a Glu instead of Lys/Arg in the first
25 position of the second copy of the motif. Similarly, YFP, which binds to one molecule of FMN, also seems to have a potentially dysfunctional binding site by substitution of Gly for Glu in the last position of the first copy of the motif. Our signature pattern includes the Lum-binding motif.
- 30 Consensus pattern: [LIVMF]-x(5)-G-[STADNQ]-[KREQIYW]-V-N-[LIVM]-E

[1] O'Kane D.J., Woodward B., Lee J., Prasher D.C. Proc. Natl. Acad. Sci. U.S.A. 88:1100-1104(1991).

[2] O'Kane D.J., Prasher D.C. Mol. Microbiol. 6:443-449(1992).

331. Lysyl oxidase putative copper-binding region signature

Lysyl oxidase (LOX) [1] is an extracellular copper-dependent enzyme that catalyzes the oxidative deamination of peptidyl lysine residues in precursors of various collagens and elastins. The deaminated lysines are then able to form aldehyde cross-links. LOX binds a single copper atom which seems to reside within an octahedral coordination complex which includes at least three histidine ligands. Four histidine residues are clustered in a central region of the enzyme. This region is thought to be involved in copper-binding and is called the 'copper-talon' [1]. This region was used as a signature pattern.

Consensus pattern: W-E-W-H-S-C-H-Q-H-Y-H

[1] Krebs C.J., Krawetz S.A. *Biochim. Biophys. Acta* 1202:7-12(1993).

332. Metallo-beta-lactamase superfamily (lactamase_B)

[1] : Neuwald AF, Liu JS, Lipman DJ, Lawrence CE, *Nucleic Acids Res* 1997;25:1665-1677. [2] Carfi A, Pares S, Duee E, Galleni M, Duez C, Frere JM, Dideberg O, *EMBO J* 1995;14:4914-4921.

333. L-lactate dehydrogenase active site (ldh1)

L-lactate dehydrogenase (EC 1.1.1.27) (LDH) [1] catalyzes the reversible NAD-dependent interconversion of pyruvate to L-lactate. In vertebrate muscles and in lactic acid bacteria it represents the final step in anaerobic glycolysis. This tetrameric enzyme is present in prokaryotic and eukaryotic organisms. Invertebrates there are three isozymes of LDH: the M form (LDH-A), found predominantly in muscle tissues; the H form (LDH-B), found in heart muscle and the X form (LDH-C), found only in the spermatozoa of mammals and birds. In birds and crocodilian eye lenses, LDH-B serves as a structural protein and is known as epsilon-crystallin [2]. L-2-hydroxyisocaproate dehydrogenase (EC 1.1.1.-) (L-hicDH) [3] catalyzes the reversible and stereospecific interconversion between 2-ketocarboxylic acids and L-2-hydroxy-carboxylic acids. L-hicDH is evolutionary related to LDH's. As a signature

for LDH's a region was selected that includes a conserved histidine which is essential to the catalytic mechanism.

Consensus pattern: [LIVMA]-G-[EQ]-H-G-[DN]-[ST] [H is the active site residue] -

5

[1] Abad-Zapatero C., Griffith J.P., Sussman J.L., Rossmann M.G. J. Mol. Biol. 198:445-467(1987).

[2] Hendriks W., Mulders J.W.M., Bibby M.A., Slingsby C., Bloemendal H., de Jong W.W. Proc. Natl. Acad. Sci. U.S.A. 85:7114-7118(1988).

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[3] Lerch H.-P., Frank R., Collins J. Gene 83:263-270(1989).

Malate dehydrogenase active site signature (ldh2)

Malate dehydrogenase (EC 1.1.1.37) (MDH) [1,2] catalyzes the interconversion of malate to oxaloacetate utilizing the NAD/NADH cofactor system. The enzyme participates in the citric acid cycle and exists in all aerobic organisms. While prokaryotic organisms contains a single form of MDH, in eukaryotic cells there are two isozymes: one which is located in the mitochondrial matrix and the other in the cytoplasm. Fungi and plants also harbor a glyoxysomal form which functions in the glyoxylate pathway. In plants chloroplast there is an additional NADP-dependent form of MDH (EC 1.1.1.82) which is essential for both the universal C3 photosynthesis (Calvin) cycle and the more specialized C4 cycle. As a signature pattern for this enzyme a region was chosen that includes two residues involved in the catalytic mechanism [3]: an aspartic acid which is involved in a proton relay mechanism, and an arginine which binds the substrate.

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Consensus pattern: [LIVM]-T-[TRKMN]-L-D-x(2)-R-[STA]-x(3)-[LIVMFY] [D and R are the active site residues]-

[1] McAlister-Henn L. Trends Biochem. Sci. 13:178-181(1988).

[2] Gietl C. Biochim. Biophys. Acta 1100:217-234(1992).

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[3] Birktoft J.J., Rhodes G., Banaszak L.J. Biochemistry 28:6065-6081(1989).

[4] Cendrin F., Chroboczek J., Zaccari G., Eisenberg H., Mevarech M. Biochemistry 32:4308-4313(1993).

334. Legume lectins signatures

Leguminous plants synthesize sugar-binding proteins which are called legume lectins [1,2]. These lectins are generally found in the seeds. The exact function of legume lectins is not known but they may be involved in the attachment of nitrogen-fixing bacteria to legumes and in the protection against pathogens. Legume lectins bind calcium and manganese (or other transition metals). Legume lectins are synthesized as precursor proteins of about 230 to 260 amino acid residues. Some legume lectins are proteolytically processed to produce two chains: beta (which corresponds to the N-terminal) and alpha (C-terminal). The lectin concanavalin A (conA) from jack bean is exceptional in that the two chains are transposed and ligated (by formation of a new peptide bond). The N-terminus of mature conA thus corresponds to that of the alpha chain and the C-terminus to the beta chain. Two signature patterns specific to legume lectins have been developed: the first is located in the C-terminal section of the beta chain and contains a conserved aspartic acid residue important for the binding of calcium and manganese; the second one is located in the N-terminal of the alpha chain.

Consensus pattern: [LIV]-[STAG]-V-[DEQV]-[FLI]-D-[ST] [D binds manganese and calcium]-

Consensus pattern: [LIV]-x-[EDQ]-[FYWKR]-V-x-[LIVF]-G-[LF]-[ST]-

[1] Sharon N., Lis H. FASEB J. 4:3198-320(1990).

[2] Lis H., Sharon N. Annu. Rev. Biochem. 55:33-37(1986).

335. CoA-ligases (ligases- CoA)

This family includes the CoA ligases Succinyl-CoA synthetase alpha: and beta chains, malate CoA ligase and ATP-citrate lyase. Some members of the family utilise ATP others use GTP.

[1] Wolodko WT, Fraser ME, James MN, Bridger WA, J Biol Chem 1994;269:10883-10890.

336. linker histone H1 and H5 family

Linker histone H1 is an essential component of chromatin structure. H1 links nucleosomes into higher order structures. Histone H1 is replaced by histone H5 in some cell types.

[1] Ramakrishnan V, Finch JT, Graziano V, Lee PL, Sweet RM, Nature

5 1993;362:219-223.

337. Lipocalin signature (lip1)

Proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids, and lipids share limited regions of sequence homology and a common tertiary structure architecture [1 to 5]. This is an eight stranded antiparallel beta-barrel with a repeated + 1 topology enclosing a internal ligand binding site [1,3]. The name 'lipocalin' has been proposed [5] for this protein family. Proteins known to belong to this family are listed below (references are only provided for recently determined sequences). - Alpha-1-microglobulin (protein HC), which seems to bind porphyrin. - Alpha-1-acid glycoprotein (orosomucoid), which can bind a remarkable array of natural and synthetic compounds [6]. - Aphrodisin which, in hamsters, functions as an aphrodisiac pheromone. - Apolipoprotein D, which probably binds heme-related compounds. - Beta-lactoglobulin, a milk protein whose physiological function appears to bind retinol. - Complement component C8 gamma chain, which seems to bind retinol [7]. - Crustacyanin [8], a protein from lobster carapace, which binds astaxanthin, a carotenoid. - Epididymal-retinoic acid binding protein (E-RABP) [9] involved in sperm maturation. - Insectacyanin, a moth bilin-binding protein, and a related butterfly bilin-binding protein (BBP). - Late Lactation protein (LALP), a milk protein from tamar wallaby [10]. - Neutrophil gelatinase-associated lipocalin (NGAL) (p25) (SV-40 induced 24p3 protein) [11]. - Odorant-binding protein (OBP), which binds odorants. - Plasma retinol-binding proteins (PRBP). - Human pregnancy-associated endometrial alpha-2 globulin. - Probasin (PB), a rat prostatic protein. - Prostaglandin D synthase (EC 5.3.99.2) (GSH-independent PGD synthetase), a lipocalin with enzymatic activity [12]. - Purpurin, a retinal protein which binds retinol and heparin. - Quiescence specific protein p20K from chicken (embryo CH21 protein). - Rodent urinary proteins (alpha-2-microglobulin), which may bind pheromones. - VNSP 1 and 2, putative pheromone transport proteins from mouse vomeronasal organ [13]. - Von Ebner's gland protein (VEGP) [14] (also called tear lipocalin), a mammalian protein which may be involved in taste recognition. - A frog olfactory protein, which may transport odorants. - A protein found in the cerebrospinal fluid of the toad Bufo

Marinus with a supposed function similar to transthyretin in transport across the blood brain barrier [15]. - Lizard's epididymal secretory protein IV (LESP IV), which could transport small hydrophobic molecules into the epididymal fluid during sperm maturation [16]. - Prokaryotic outer-membrane protein blc [17]. The sequences of most members of the family, the core or kernal lipocalins, are characterized by three short conserved stretches of residues [3,18]. Others, the outlier lipocalin group, share only one or two of these [3,18]. A signature pattern was built around the first, common to all outlier and kernallipocalins, which occurs near the start of the first beta-strand.

Consensus pattern: [DENG]-x-[DENQGSTARK]-x(0,2)-[DENQARK]-[LIVFY]-{CP}-G-{C}-W-[FYWLRH]-x-[LIVMTA]-

Note: it is suggested, on the basis of similarities of structure, function, and sequence, that this family forms an overall superfamily, called the calycins, with the avidin/streptavidin <PDOC00499> and the cytosolic fatty- acid binding proteins <PDOC00188> families [3,19]

[1] Cowan S.W., Newcomer M.E., Jones T.A. Proteins 8:44-61(1990).

[2] Igaraishi M., Nagata A., Toh H., Urade H., Hayaishi N. Proc. Natl. Acad. Sci. U.S.A. 89:5376-5380(1992).

[3] Flower D.R., North A.C.T., Attwood T.K. Protein Sci. 2:753-761(1993).

[4] Godovac-Zimmermann J. Trends Biochem. Sci. 13:64-66(1988).

[5] Pervaiz S., Brew K. FASEB J. 1:209-214(1987).

[6] Kremer J.M.H., Wilting J., Janssen L.H.M. Pharmacol. Rev. 40:1-47(1989).

[7] Haeffliger J.-A., Peitsch M.C., Jenne D., Tschoep J. Mol. Immunol. 28:123-131(1991).

[8] Keen J.N., Caceres I., Eliopoulos E.E., Zagalsky P.F., Findlay J.B.C. Eur. J. Biochem. 197:407-417(1991).

[9] Newcomer M.E. Structure 1:7-18(1993).

[10] Collet C., Joseph R. Biochim. Biophys. Acta 1167:219-222(1993).

[11] Kjeldsen L., Johnsen A.H., Sengelov H., Borregaard N. J. Biol. Chem. 268:10425-10432(1993).

[12] Peitsch M.C., Boguski M.S. Trends Biochem. Sci. 16:363-363(1991).

[13] Miyawaki A., Matsushita Y.R., Ryo Y., Mikoshiba T. EMBO J. 13:5835-5842(1994).

[14] Kock K., Ahlers C., Schmale H. Eur. J. Biochem. 221:905-916(1994).

[15] Achen M.G., Harms P.J., Thomas T., Richardson S.J., Wettenhall R.E.H., Schreiber G. J. Biol. Chem. 267:23170-23174(1992).

[16] Morel L., Dufarre J.-P., Depeiges A. J. Biol. Chem. 268:10274-10281(1993).

[17] Bishop R.E., Penfold S.S., Frost L.S., Holtje J.V., Weiner J.H. J. Biol. Chem. 270:23097-23103(1995).

[18] Flower D.R., North A.C.T., Attwood T.K. Biochem. Biophys. Res. Commun. 180:69-74(1991).

[19] Flower D.R. FEBS Lett. 333:99-102(1993).

Cytosolic fatty-acid binding proteins signature (lip2)

A number of low molecular weight proteins which bind fatty acids and other organic anions

are present in the cytosol [1,2]. Most of them are structurally related and have probably diverged from a common ancestor. This structure is a ten stranded antiparallel beta-barrel, albeit with a wide discontinuity between the fourth and fifth strands, with a repeated + 1 topology enclosing an internal ligand binding site [2,7]. Proteins known to belong to this family include: - Six, tissue-specific, types of fatty acid binding proteins (FABPs) found in liver, intestine, heart, epidermal, adipocyte, brain/retina. Heart FABP is also known as mammary-derived growth inhibitor (MDGI), a protein that reversibly inhibits proliferation of mammary carcinoma cells. Epidermal FABP is also known as psoriasis-associated FABP [3]. - Insect muscle fatty acid-binding proteins. - Testis lipid binding protein (TLBP). - Cellular retinol-binding proteins I and II (CRBP). - Cellular retinoic acid-binding protein (CRABP). - Gastrotropin, an ileal protein which stimulates gastric acid and pepsinogen secretion. It seems that gastrotropin binds to bile salts and bilirubins. - Fatty acid binding proteins MFB1 and MFB2 from the midgut of the insect Manduca sexta [4]. In addition to the above cytosolic proteins, this family also includes: - Myelin P2 protein, which may be a lipid transport protein in Schwann cells. P2 is associated with the lipid bilayer of myelin. - Schistosoma mansoni protein Sm14 [5] which seems to be involved in the transport of fatty acids. - Ascaris suum p18 a secreted protein that may play a role in sequestering potentially toxic fatty acids and their peroxidation products or that may be involved in the maintenance of the impermeable lipid layer of the eggshell. - Hypothetical fatty acid-binding proteins F40F4.2, F40F4.3, F40F4.4 and ZK742.5 from Caenorhabditis elegans. As a signature pattern for these proteins a segment from the N-terminal extremity was used.

Consensus pattern: [GSAIVK]-x-[FYW]-x-[LIVMF]-x(4)-[NHG]-[FY]-[DE]-x-[LIVMFY]-[LIVM]-x(2)-[LIVMAKR]-

Note: it is suggested, on the basis of similarities of structure, function, and sequence, that this family forms an overall superfamily, called the calycins, with the lipocalin <PDOC00187> and avidin/streptavidin <PDOC00499> families [6,7].

- 5 [1] Bernier I., Jolles P. Biochimie 69:1127-1152(1987).
- [2] Veerkamp J.H., Peeters R.A., Maatman R.G.H.J. Biochim. Biophys. Acta 1081:1-24(1991).
- [3] Siegenthaler G., Hotz R., Chatellard-Gruaz D., Didierjean L., Hellman U., Saurat J.-H. Biochem. J. 302:363-371(1994).
- 10 [4] Smith A.F., Tsuchida K., Hanneman E., Suzuki T.C., Wells M.A. J. Biol. Chem. 267:380-384(1992).
- [5] Moser D., Tendler M., Griffiths G., Klinkert M.-Q. J. Biol. Chem. 266:8447-8454(1991).
- [6] Flower D.R., North A.C.T., Attwood T.K. Protein Sci. 2:753-761(1993).
- [7] Flower D.R. FEBS Lett. 333:99-102(1993).

338. Lipoxygenases iron-binding region signatures

- Lipoxygenases (EC 1.13.11.-) are a class of iron-containing dioxygenases which catalyzes the hydroperoxidation of lipids, containing a cis,cis-1,4-pentadiene structure. They are common
- 20 in plants where they may be involved in a number of diverse aspects of plant physiology including growth and development, pest resistance, and senescence or responses to wounding [1]. In mammals a number of lipoxygenases isozymes are involved in the metabolism of prostaglandins and leukotrienes [2]. Sequence data is available for the following lipoxygenases: - Plant lipoxygenases (EC 1.13.11.12). Plants express a variety of cytosolic
 - 25 isozymes as well as what seems [3] to be a chloroplast isozyme. - Mammalian arachidonate 5-lipoxygenase (EC 1.13.11.34). - Mammalian arachidonate 12-lipoxygenase (EC 1.13.11.31). - Mammalian erythroid cell-specific 15-lipoxygenase (EC 1.13.11.33). The iron atom in lipoxygenases is bound by four ligands, three of which are histidine residues [4]. Six histidines are conserved in all lipoxygenase sequences, five of them are found clustered in a
 - 30 stretch of 40 amino acids. This region contains two of the three zinc-ligands; the other histidines have been shown [5] to be important for the activity of lipoxygenases. As signatures for this family of enzymes two patterns in the region of the histidine cluster were selected. The first pattern contains the first three conserved histidines and the second pattern includes the fourth and the fifth.

Consensus pattern: H-[EQ]-x(3)-H-x-[LM]-[NQRC]-[GST]-H-[LIVMSTAC](3)-E [The second and third H's bind iron]-

Consensus pattern: [LIVMA]-H-P-[LIVM]-x-[KRQ]-[LIVMF](2)-x-[AP]-H-

5

[1] Vick B.A., Zimmerman D.C. (In) Biochemistry of plants: A comprehensive treatise, Stumpf P.K., Ed., Vol. 9, pp.53-90, Academic Press, New-York, (1987).

[2] Needleman P., Turk J., Jakschik B.A., Morrison A.R., Lefkowitz J.B. Annu. Rev. Biochem. 55:69-102(1986).

10 [3] Peng Y.L., Shirano Y., Ohta H., Hibino T., Tanaka K., Shibata D. J. Biol. Chem. 269:3755-3761(1994).

[4] Boyington J.C., Gaffney B.J., Amzel L.M. Science 260:1482-1486(1993).

[5] Steczko J., Donoho G.P., Clemens J.C., Dixon J.E., Axelrod B. Biochemistry 31:4053-4057(1992).

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339. Fumarate lyases signature (lyase_1)

A number of enzymes, belonging to the lyase class, for which fumarate is a substrate have been shown [1,2] to share a short conserved sequence around a methionine which is probably involved in the catalytic activity of this type of enzymes. These enzymes are: - Fumarase (EC 4.2.1.2) (fumarate hydratase), which catalyzes the reversible hydration of fumarate to L-malate. There seem to be 2 classes of fumarases: class I are thermolabile dimeric enzymes (as for example: Escherichia coli fumC); class II enzymes are thermostable and tetrameric and are found in prokaryotes (as for example: Escherichia coli fumA and fumB) as well as in

25 eukaryotes. The sequence of the two classes of fumarases are not closely related. - Aspartate ammonia-lyase (EC 4.3.1.1) (aspartase), which catalyzes the reversible conversion of aspartate to fumarate and ammonia. This reaction is analogous to that catalyzed by fumarase, except that ammonia rather than water is involved in the trans-elimination reaction. - Argininosuccinase (EC 4.3.2.1) (argininosuccinate lyase), which catalyzes the formation of arginine and fumarate from argininosuccinate, the last step in the biosynthesis of arginine. - Adenylosuccinase (EC 4.3.2.2) (adenylosuccinate lyase) [3], which catalyzes the eight step in the de novo biosynthesis of purines, the formation of 5'-phosphoribosyl-5-amino-4-imidazolecarboxamide and fumarate from 1-(5'-phosphoribosyl)-4-(N-succino-carboxamide). That enzyme can also catalyzes the formation of fumarate and AMP from adenylosuccinate. -

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Pseudomonas putida 3-carboxy-cis,cis-muconate cycloisomerase (EC 5.5.1.2) (3-carboxymuconate lactonizing enzyme) (gene *pcaB*) [4], an enzyme involved in aromatic acids catabolism

5 Consensus pattern: G-S-x(2)-M-x(2)-K-x-N-

[1] Woods S.A., Shwartzbach S.D., Guest J.R. Biochim. Biophys. Acta 954:14-26(1988).

[2] Woods S.A., Miles J.S., Guest J.R. FEMS Microbiol. Lett. 51:181-186(1988).

[3] Zalkin H., Dixon J.E. Prog. Nucleic Acid Res. Mol. Biol. 42:259-287(1992).

10 [4] Williams S.E., Woolridge E.M., Ransom S.C., Landro J.A., Babbitt P.C., Kozarich J.W. Biochemistry 31:9768-9776(1992).

340. MCM family signature and profile

- 15 Proteins shown to be required for the initiation of eukaryotic DNA replication share a highly conserved domain of about 210 amino-acid residues [1,2,3]. The latter shows some similarities [4] with that of various other families of DNA-dependent ATPases. Eukaryotes seem to possess a family of six proteins that contain this domain. They were first identified in yeast where most of them have a direct role in the initiation of chromosomal DNA replication
- 20 by interacting directly with autonomously replicating sequences (ARS). They were thus called 'minichromosome maintenance proteins' with gene symbols prefixed by MCM. These six proteins are: - MCM2, also known as *cdc19* (in *S.pombe*) [E1]. - MCM3, also known as DNA polymerase alpha holoenzyme-associated protein P1, RLF beta subunit or ROA. - MCM4, also known as *CDC54*, *cdc21* (in *S.pombe*) or *dpa* (in *Drosophila*). - MCM5, also
- 25 known as *CDC46* or *nda4* (in *S.pombe*). - MCM6, also known as *mis5* (in *S.pombe*). - MCM7, also known as *CDC47* or *Prolifera* (in *A.thaliana*). This family is also present in archaebacteria. In *Methanococcus jannaschii* there are four members: MJ0363, MJ0961, MJ1489 and MJEL13. The presence of a putative ATP-binding domain implies that these proteins maybe involved in an ATP-consuming step in the initiation of DNA replication in
- 30 eukaryotes. As a signature pattern, a perfectly conserved region was selected that represents a special version of the B motif found in ATP-binding proteins.

Consensus pattern: G-[IVT]-[LVAC](2)-[IVT]-D-[DE]-[FL]-[DNST]

- [1] Coxon A., Maundrell K., Kearsey S.E. *Nucleic Acids Res.* 20:5571-5577(1992).
 [2] Hu B., Burkhardt R., Schulte D., Musahl C., Knippers R. *Nucleic Acids Res.* 21:5289-5293(1993).
 [3] Tye B.-K. *Trends Cell Biol.* 4:160-166(1994).
 5 [4] Koonin E.V. *Nucleic Acids Res.* 21:2541-2547(1993).

341. Macrophage migration inhibitory factor family signature (MIF)

- A protein called macrophage migration inhibitory factor (MIF) [1] seems to exert an
 10 important role in host inflammatory responses. It play a pivotal role in the host response to
 endotoxic shock and appears to serve as a pituitary "stress" hormone that regulates systemic
 inflammatory responses. MIF is a secreted protein of 115 residues which is not processed
 from a larger precursor. D-dopachrome tautomerase [2] is a mammalian cytoplasmic enzyme
 involved in melanin biosynthesis and that tautomerizes D-dopachrome with concomitant
 15 decarboxylation to give 5,6-dihydroxyindole (DHI). It is a protein of 117 residues highly
 related to MIF. It must be noted that MIF binds glutathione and has been said to be related to
 glutathione S-transferases. This assertion has been later disproved [3]. As a signature pattern
 for these proteins, a conserved region was selected located in the central section.
 20 Consensus pattern: [DE]-P-C-A-x(3)-[LIVM]-x-S-I-G-x-[LIVM]-G-

- [1] Bucala R. *Immunol. Lett.* 43:23-26(1994).
 [2] Odh G., Hindemith A., Rosengren A.-M., Rosengren E., Rorsman H. *Biochem. Biophys. Res. Commun.* 197:619-624(1993).
 25 [3] Pearson W.R. *Protein Sci.* 3:525-527(1994).

342. MIP family signature

- Recently the sequence of a number of different proteins, that all seem to be transmembrane
 30 channel proteins, has been found to be highly related [1 to 4]. These proteins are listed below.
 - Mammalian major intrinsic protein (MIP). MIP is the major component of lens fiber gap
 junctions. Gap junctions mediate direct exchange of ions and small molecule from one cell to
 another. - Mammalian aquaporins [5]. These proteins form water-specific channels that
 provide the plasma membranes of red cells and kidney proximal and collecting tubules with

- high permeability to water, thereby permitting water to move in the direction of an osmotic gradient. - Soybean nodulin-26, a major component of the peribacteroid membrane induced during nodulation in legume roots after *Rhizobium* infection. - Plants tonoplast intrinsic proteins (TIP). There are various isoforms of TIP: alpha (seed), gamma, Rt (root), and Wsi (water-stress induced). These proteins may allow the diffusion of water, amino acids and/or peptides from the tonoplast interior to the cytoplasm. - Bacterial glycerol facilitator protein (gene glpF), which facilitates the movement of glycerol across the cytoplasmic membrane. - *Salmonella typhimurium* propanediol diffusion facilitator (gene pduF). - Yeast FPS1, a glycerol uptake/efflux facilitator protein. - *Drosophila* neurogenic protein 'big brain' (bib).
- 10 This protein may mediate intercellular communication; it may functions by allowing the transport of certain molecules(s) and thereby sending a signal for an exodermal cell to become an epidermoblast instead of a neuroblast. - Yeast hypothetical protein YFL054c. - A hypothetical protein from the pepX region of *Lactococcus lactis*. The MIP family proteins seem to contain six transmembrane segments. Computer analysis shows that these protein
- 15 probably arose by a tandem, intragenic duplication event from an ancestral protein that contained three transmembrane segments. As a signature pattern a well conserved region was selected which is located in a probable cytoplasmic loop between the second and third transmembrane regions.
- 20 Consensus pattern: [HNQA]-x-N-P-[STA]-[LIVMF]-[ST]-[LIVMF]-[GSTAFY]-

[1] Reizer J., Reizer A., Saier M.H. Jr. *CRC Crit. Rev. Biochem.* 28:235-257(1993).

[2] Baker M.E., Saier M.H. Jr. *Cell* 60:185-186(1990).

[3] Pao G.M., Wu L.-F., Johnson K.D., Hoeft H., Chrispeels M.J., Sweet G., Sandal N.N.,

25 Saier M.H. Jr. *Mol. Microbiol.* 5:33-37(1991).

[4] Wistow G.J., Pisano M.M., Chepelinsky A.B. *Trends Biochem. Sci.* 16:170-171(1991).

[5] Chrispeels M.J., Agre P. *Trends Biochem. Sci.* 19:421-425(1994).

30 343. Mandelate racemase / muconate lactonizing enzyme family signatures

Mandelate racemase (EC 5.1.2.2) (MR) and muconate lactonizing enzyme (EC 5.5.1.1)

(MLE) are two bacterial enzymes involved in aromatic acid catabolism. They catalyze mechanistically distinct reactions yet they are related at the level of their primary, quaternary (homooctamer) and tertiary structures [1,2]. A number of other proteins also seem to be

evolutionary related to these two enzymes. These are: - The various plasmid-encoded chloromuconate cycloisomerases (EC 5.5.1.7). - *Escherichia coli* protein *rspA* [3], *rspA* seems to be involved in the degradation of homoserine lactone (HSL) or of one of its metabolite. - *Escherichia coli* hypothetical protein *ycjG*. - *Escherichia coli* hypothetical protein *yidU*. - A hypothetical protein from *Streptomyces ambofaciens* [4]. Two signature patterns have been developed for these enzymes; both contain conserved acidic residues. The second pattern contains an aspartate and a glutamate which are ligands for either a magnesium ion (in MR) or a manganese ion (inMLE).

10 Consensus pattern: A-x-[SAGCN]-[SAG]-[LIVM]-[DEQ]-x-A-[LA]-x-[DE]-[LIA]-x-[GA]-[KRO]-x(4)-[PSA]-[LIV]-x(2)-L-[LIVMF]-G-

Consensus pattern: [LIVF]-x(2)-D-x-[NH]-x(7)-[ACL]-x(6)-[LIVMF]-x(7)-[LIVM]-E-[DENQ]-P [D and E bind a divalent metal ion]-

- 15 [1] Neidhart D.J., Kenyon G.L., Gerlt J.A., Petsko G.A. Nature 347:692-694(1990).
 [2] Petsko G.A., Kenyon G.L., Gerlt J.A., Ringe D., Kozarich J.W. Trends Biochem. Sci. 18:372-376(1993).
 [3] Huisman G.W., Kolter R. Science 265:537-539(1994).
 [4] Schneider D., Aigle B., Leblond P., Simonet J.M., Decaris B. J. Gen. Microbiol.
 20 139:2559-2567(1993).

344. Merozoite Surface Antigen 2 (MSA-2) family

Thomas AW, Carr DA, Carter JM, Lyon JA, Mol Biochem Parasitol 1990;43:211-

25 220.

345. MSP (Major sperm protein) domain.

- Major sperm proteins are involved in sperm motility. These proteins oligomerise to
 30 form filaments. Partial matches to this domain are also found in other non MSP proteins.
 These include [Swiss:P40075](#) and [Swiss:P34593](#).

[1] Bullock TL, Roberts TM, Stewart M, J Mol Biol 1996;263:284-296. [2] King KL, Stewart M, Roberts TM, Seavy M, J Cell Sci 1992;101:847-857.

346. (Matrix) Viral matrix protein. Found in Morbillivirus and paramyxovirus, pneumovirus.
Number of members: 105

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347. O-methyltransferase (methyltransf)

This family includes a range of O-methyltransferases. These enzymes utilise S-adenosyl methionine.

[1] Keller NP, Dischinger HC, Bhatnagar D, Cleveland TE, Ullah AH, Appl Environ
10 Microbiol 1993;59:479-484.

348. Magnesium chelatase, subunit ChII

Magnesium-chelatase is a three-component enzyme that catalyses the insertion of
15 Mg²⁺ into protoporphyrin IX. This is the first unique step in the synthesis of
(bacterio)chlorophyll. Due to this, it is thought that Mg-chelatase has an important role in
channeling inter- mediates into the (bacterio)chlorophyll branch in response to conditions
suitable for photosynthetic growth. ChII and BchD have molecular weight between 38-42
kDa.

[1] Walker CJ, Willows RD, Biochem J 1997;327:321-333. [2] Petersen BL, Jensen
20 PE, Gibson LC, Stummann BM, Hunter CN, Henningsen KW, J Bacteriol 1998;180:699-704.

349. Plasmid recombination enzyme (Mob_Pre)

25 With some plasmids, recombination can occur in a site specific manner that is
independent of RecA. In such cases, the recombination event requires another protein called
Pre. Pre is a plasmid recombination enzyme. This protein is: also known as Mob (conjugative
mobilization).

[1] Priebe SD, Lacks SA, J Bacteriol 1989;171:4778-4784.
30

350. Monooxygenase

This family includes diverse enzymes that utilise FAD.

[1] Gatti DL, Palfey BA, Lah MS, Entsch B, Massey V, Ballou DP, Ludwig ML, Science 1994;266:110-114.

5 351. Mov34 family

Members of this family are found in proteasome regulatory subunits, eukaryotic initiation factor 3 (eIF3) subunits and regulators of transcription factors.

[1] Aravind L, Ponting CP, Protein Sci 1998;7:1250-1254. [2] Hershey JW, Asano K, Naranda T, Vornlocher HP, Hanachi P, Merrick WC, Biochimie 1996;78:903-907.

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352. Myc amino-terminal region (Myc_N_term)

The myc family belongs to the basic helix-loop-helix leucine zipper class of transcription factors, see HLH. Myc forms a heterodimer with Max, and this complex regulates cell growth through direct activation of genes involved in cell replication [2].

[1] Facchini LM, Penn LZ, FASEB J 1998;12:633-651. [2] Grandori C, Eisenman RN, Trends Biochem Sci 1997;22:177-181.

20 353. (Metallothio_2) Metallothionein. Members of this family are metallothioneins. These proteins are cysteine rich proteins that bind to heavy metals. Members of this family appear to be closest to Class II metallothioneins, seed metalthio. Number of members: 55

[1] Medline: 98267202. Characterization of gene repertoires at mature stage of citrus fruits through random sequencing and analysis of redundant metallothionein-like genes expressed during fruit development. Moriguchi T, Kita M, Hisada S, Endo-Inagaki T, Omura M; Gene 1998;211:221-227.

30 354. MAGE family

The MAGE (melanoma antigen-encoding gene) family are expressed in a wide variety of tumors but not in normal cells, with the exception of the male germ cells, placenta, and, possibly,

cells of the developing embryo. The cellular function of this family is unknown.

[1] McCurdy DK, Tai LQ, Nguyen J, Wang Z, Yang HM, Udar N,

5 Naiem F, Concannon P, Gatti RA; Mol Genet Metab 1998;63:3-13.

355. Malic enzymes signature. Malic enzymes, or malate oxidoreductases, catalyze the oxidative decarboxylation of malate into pyruvate important for a wide range of metabolic pathways. There are three related forms of malic enzyme [1,2,3]: - NAD-dependent malic enzyme (EC 1.1.1.38), which uses preferentially NAD and has the ability to decarboxylate oxaloacetate (OAA). It is found in bacteria and insects. - NAD-dependent malic enzyme (EC 1.1.1.39), which uses preferentially NAD and is unable to decarboxylate OAA. It is found in the mitochondrial matrix of plants and is a heterodimer of highly related subunits. - NADP-dependent malic enzyme (EC 1.1.1.40), which has a preference for NADP and has the ability to decarboxylate OAA. This form has been found in fungi, animals and plants. In mammals, there are two isozymes: one, mitochondrial and the other, cytosolic. Plants also have two isozymes: chloroplastic and cytosolic. There are two other proteins which are closely structurally related to malicenzymes: - Escherichia coli protein sfcA, whose function is not yet known but which could be an NAD or NADP-dependent malic enzyme. - Yeast hypothetical protein YKL029c, a probable malic enzyme. There are three well conserved regions in the enzyme sequences. Two of them seem to be involved in binding NAD or NADP. The significance of the third one, located in the central part of the enzymes, is not yet known. This region has been developed as a signature pattern for these enzymes.

Consensus pattern: F-x-[DV]-D-x(2)-G-T-[GSA]-x-[IV]-x-[LIVMA]-[GAST](2)-[LIVMF](2)-

[1] Artus N.N., Edwards G.E. FEBS Lett. 182:225-233(1985). [2] Loeber G., Infante A.A., Maurer-Fogy I., Krystek E., Dworkin M.B. J. Biol. Chem. 266:3016-3021(1991). [3] Long J.J., Wang J.-L., Berry J.O. J. Biol. Chem. 269:2827-2833(1994).

356. (matrixin)

Matrixins cysteine switch (aka peptidase_M10)

Mammalian extracellular matrix metalloproteinases (EC 3.4.24.-), also known as matrixins [1] (see <PDOC00129>), are zinc-dependent enzymes. They are secreted by cells in an inactive form (zymogen) that differs from the mature enzyme by the presence of an N-terminal propeptide. A highly conserved octapeptide is found two residues downstream of the C-terminal end of the propeptide. This region has been shown to be involved in autoinhibition of matrixins [2,3]; a cysteine within the octapeptide chelates the active site zinc ion, thus inhibiting the enzyme. This region has been called the 'cysteine switch' or 'autoinhibitor region'.

A cysteine switch has been found in the following zinc proteases:

- MMP-1 (EC 3.4.24.7) (interstitial collagenase).
- MMP-2 (EC 3.4.24.24) (72 Kd gelatinase).
- MMP-3 (EC 3.4.24.17) (stromelysin-1).
- MMP-7 (EC 3.4.24.23) (matrilysin).
- MMP-8 (EC 3.4.24.34) (neutrophil collagenase).
- MMP-9 (EC 3.4.24.35) (92 Kd gelatinase).
- MMP-10 (EC 3.4.24.22) (stromelysin-2).
- MMP-11 (EC 3.4.24.-) (stromelysin-3).
- MMP-12 (EC 3.4.24.65) (macrophage metalloelastase).
- MMP-13 (EC 3.4.24.-) (collagenase 3).
- MMP-14 (EC 3.4.24.-) (membrane-type matrix metalloproteinase 1).
- MMP-15 (EC 3.4.24.-) (membrane-type matrix metalloproteinase 2).
- MMP-16 (EC 3.4.24.-) (membrane-type matrix metalloproteinase 3).
- Sea urchin hatching enzyme (EC 3.4.24.12) (envelysin) [4].
- Chlamydomonas reinhardtii gamete lytic enzyme (GLE) [5].

Consensus pattern P-R-C-[GN]-x-P-[DR]-[LIVSAPKO] [C chelates the zinc ion] Sequences known to belong to this class detected by the pattern ALL, except for cat MMP-7 and mouse MMP-11.

[1] Woessner J. Jr. FASEB J. 5:2145-2154(1991).

[2] Sanchez-Lopez R., Nicholson R., Gesnel M.C., Matrisian L.M., Breathnach R. J. Biol. Chem. 263:11892-11899(1988).

5 [3] Park A.J., Matrisian L.M., Kells A.F., Pearson R., Yuan Z., Navre M. J. Biol. Chem. 266:1584-1590(1991).

[4] Lepage T., Gache C. EMBO J. 9:3003-3012(1990).

[5] Kinoshita T., Fukuzawa H., Shimada T., Saito T., Matsuda Y. Proc. Natl. Acad. Sci. U.S.A. 89:4693-4697(1992).

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357. Vertebrate metallothioneins signature (metalthio)

15 Metallothioneins (MT) [1,2,3] are small proteins which bind heavy metals such as zinc, copper, cadmium, nickel, etc., through clusters of thiolate bonds. MT's occur throughout the animal kingdom and are also found in higher plants, fungi and some prokaryotes. On the basis of structural relationships MT's have been subdivided into three classes. Class I includes mammalian MT's as well as MT's from crustacean and molluscs, but with clearly related primary structure. Class II groups together MT's from various species such as sea urchins, fungi, insects and cyanobacteria which display none or only very distant correspondence to
20 class I MT's. Class III MT's are atypical polypeptides containing gamma-glutamylcysteinyl units. Vertebrate class I MT's are proteins of 60 to 68 amino acid residues, 20 of these residues are cysteines that bind to 7 bivalent metal ions. As a signature pattern a region that spans 19 residues and which contains seven of the metal-binding cysteines was chosen, this region is located in the N-terminal section of class-I MT's.

25

Consensus pattern: C-x-C-[GSTAP]-x(2)-C-x-C-x(2)-C-x-C-x(2)-C-x-K-

[1] Hamer D.H. Annu. Rev. Biochem. 55:913-951(1986).

[2] Kagi J.H.R., Schaffer A. Biochemistry 27:8509-8515(1988).

30 [3] Binz P.-A. Thesis, 1996, University of Zurich.

358. Mitochondrial energy transfer proteins signature (mito_carr)

Different types of substrate carrier proteins involved in energy transfer are found in the inner mitochondrial membrane [1 to 5]. These are: - The ADP,ATP carrier protein (AAC) (ADP/ATP translocase) which exports ATP into the cytosol and imports ADP into the mitochondrial matrix. The sequence of AAC has been obtained from various mammalian, plant and fungal species. - The 2-oxoglutarate/malate carrier protein (OGCP), which exports 2-oxoglutarate into the cytosol and imports malate or other dicarboxylic acids into the mitochondrial matrix. This protein plays an important role in several metabolic processes such as the malate/aspartate and the oxoglutarate/isocitrate shuttles. - The phosphate carrier protein, which transports phosphate groups from the cytosol into the mitochondrial matrix. - The brown fat uncoupling protein (UCP) which dissipates oxidative energy into heat by transporting protons from the cytosol into the mitochondrial matrix. - The tricarboxylate transport protein (or citrate transport protein) which is involved in citrate-H⁺/malate exchange. It is important for the bioenergetics of hepatic cells as it provides a carbon source for fatty acid and sterol biosyntheses, and NAD for the glycolytic pathway. - The Grave's disease carrier protein (GDC), a protein of unknown function recognized by IgG in patients with active Grave's disease. - Yeast mitochondrial proteins MRS3 and MRS4. The exact function of these proteins is not known. They suppress a mitochondrial splice defect in the first intron of the COB gene and may act as carriers, exerting their suppressor activity by modulating solute concentrations in the mitochondrion. - Yeast mitochondrial FAD carrier protein (gene FLX1). - Yeast protein ACR1 [6], which seems essential for acetyl-CoA synthetase activity. - Yeast protein PET8. - Yeast protein PMT. - Yeast protein RIM2. - Yeast protein YHM1/SHM1. - Yeast protein YMC1. - Yeast protein YMC2. - Yeast hypothetical proteins YBR291c, YEL006w, YER053c, YFR045w, YHR002w, and YIL006w. - *Caenorhabditis elegans* hypothetical protein K11H3.3. Two other proteins have been found to belong to this family, yet are not localized in the mitochondrial inner membrane: - Maize amyloplast Brittle-1 protein. This protein, found in the endosperm of kernels, could play a role in amyloplast membrane transport. - *Candida boidinii* peroxisomal membrane protein PMP47 [7]. PMP47 is an integral membrane protein of the peroxisome and it may play a role as a transporter. These proteins all seem to be evolutionary related. Structurally, they consist of three tandem repeats of a domain of approximately one hundred residues. Each of these domains contains two transmembrane regions. As a signature pattern, one of the most conserved regions in the repeated domain was selected, located just after the first transmembrane region.

Consensus pattern: P-x-[DE]-x-[LIVAT]-[RK]-x-[LRH]-[LIVMFY]-[QGAIVM]-

- [1] Klingenberg M. Trends Biochem. Sci. 15:108-112(1990).
 - [2] Walker J.E. Curr. Opin. Struct. Biol. 2:519-526(1992).
 - 5 [3] Kuan J., Saier M.H. Jr. CRC Crit. Rev. Biochem. 28:209-233(1993).
 - [4] Kuan J., Saier M.H. Jr. Res. Microbiol. 144:671-672(1993).
 - [5] Nelson D.R., Lawson J.E., Klingenberg M., Douglas M.G. J. Mol. Biol. 230:1159-1170(1993).
 - [6] Palmieri F. FEBS Lett. 346:48-54(1994).
 - 10 [7] Jank B., Habermann B., Schweyen R.J., Link T.A. Trends Biochem. Sci. 18:427-428(1993).
359. Prokaryotic molybdopterin oxidoreductases signatures (molybdopterin)
- 15 A number of different prokaryotic oxidoreductases that require and bind amolybdopterin cofactor have been shown [1,2,3] to share a number of regions of sequence similarity. These enzymes are: - Escherichia coli respiratory nitrate reductase (EC 1.7.99.4). This enzyme complex allows the bacteria to use nitrate as an electron acceptor during anaerobic growth. The enzyme is composed of three different chains: alpha, beta and gamma. The alpha chain
 - 20 (gene narG) is the molybdopterin-binding subunit. Escherichia coli encodes for a second, closely related, nitrate reductase complex which also contains a molybdopterin-binding alpha chain (gene narX). - Escherichia coli anaerobic dimethyl sulfoxide reductase (DMSO reductase). DMSO reductase is the terminal reductase during anaerobic growth on various sulfoxide and N-oxide compounds. DMSO reductase is composed of three chains: A, B and
 - 25 C. The A chain (gene dmsA) binds molybdopterin. - Escherichia coli biotin sulfoxide reductases (genes bisC and bisZ). This enzyme reduces a spontaneous oxidation product of biotin, BDS, back to biotin. It may serve as a scavenger, allowing the cell to use biotin sulfoxide as a biotin source. - Methanobacterium formicicum formate dehydrogenase (EC 1.2.1.2). The alpha chain (gene fdhA) of this dimeric enzyme binds a molybdopterin cofactor.
 - 30 - Escherichia coli formate dehydrogenases -H (gene fdhF), -N (gene fdnG) and -O (gene fdOG). These enzymes are responsible for the oxidation of formate to carbon dioxide. In addition to molybdopterin, the alpha (catalytic) subunit also contains an active site, selenocysteine. - Wolinella succinogenes polysulfide reductase chain. This enzyme is a component of the phosphorylative electron transport system with polysulfide as the terminal

acceptor. It is composed of three chains: A, B and C. The A chain (gene *psrA*) binds molybdopterin. - *Salmonella typhimurium* thiosulfate reductase (gene *phsA*). - *Escherichia coli* trimethylamine-N-oxide reductase (EC 1.6.6.9) (gene *torA*) [4]. - Nitrate reductase (EC 1.7.99.4) from *Klebsiella pneumoniae* (gene *nasA*), *Alcaligenes eutrophus*, *Escherichia coli*,
 5 *Rhodobacter sphaeroides*, *Thiosphaera pantotropha* (gene *napA*), and *Synechococcus* PCC 7942 (gene *narB*). These proteins range from 715 amino acids (*fdhF*) to 1246 amino acids (*narZ*) insize. Three signature patterns for these enzymes were derived. The first is based on a conserved region in the N-terminal section and contains two cysteine residues perhaps
 10 involved in binding the molybdopterin cofactor. It should be noted that this region is not present in *bisC*. The second pattern is derived from a conserved region located in the central part of these enzymes.

Consensus pattern: [STAN]-x-[CH]-x(2,3)-C-[STAG]-[GSTVMF]-x-C-x-[LIVMFYW]-x-[LIVMA]-x(3,4)-[DENQKHT]-

15 Consensus pattern: [STA]-x-[STAC](2)-x(2)-[STA]-D-[LIVMY](2)-L-P-x-[STAC](2)- x(2)-E-

Consensus pattern: A-x(3)-[GDT]-I-x-[DNQTK]-x-[DEA]-x-[LIVM]-x-[LIVMC]-x- [NS]-x(2)-[GS]-x(5)-A-x-[LIVM]-[ST]-

- 20 [1] Wootton J.C., Nicolson R.E., Cock J.M., Walters D.E., Burke J.F., Doyle W.A., Bray R.C. *Biochim. Biophys. Acta* 1057:157-185(1991).
 [2] Bilous P.T., Cole S.T., Anderson W.F., Weiner J.H. *Mol. Microbiol.* 2:785-795(1988).
 [3] Trieber C.A., Rothery R.A., Weiner J.H. *J. Biol. Chem.* 269:7103-7109(1994).
 [4] Mejean V., Lobbi-Nivol C., Lepelletier M., Giordano G., Chippaux M., Pascal M.-C.
 25 *Mol. Microbiol.* 11:1169-1179(1994).

360. Bacterial mutT domain signature

The bacterial mutT protein is involved in the GO system [1] responsible for removing an
 30 oxidatively damaged form of guanine (8-hydroxyguanine or 7,8-dihydro-8-oxoguanine) from DNA and the nucleotide pool. 8-oxo-dGTP is inserted opposite to dA and dC residues of template DNA with almost equal efficiency thus leading to A.T to G.C transversions. MutT specifically degrades 8-oxo-dGTP to the monophosphate with the concomitant release of pyrophosphate. MutT is a small protein of about 12 to 15 Kd. It has been shown [2,3] that a

region of about 40 amino acid residues, which is found in the N-terminal part of mutT, can also be found in a variety of other prokaryotic, viral, and eukaryotic proteins. These proteins are:

- *Streptomyces pneumoniae* mutX.
- A mutT homolog from plasmid pSAM2 of *Streptomyces ambifaciens*.
- *Bartonella bacilliformis* invasion protein A (gene invA).
- *Escherichia coli* dATP pyrophosphohydrolase.
- Protein D250 from African swine fever viruses.
- Proteins D9 and D10 from a variety of poxviruses.
- Mammalian 7,8-dihydro-8-oxoguanine triphosphatase (EC 3.1.6.-) [4].
- Mammalian diadenosine 5',5'''-P₁,P₄-tetrphosphate asymmetrical hydrolase (Ap₄Aase) (EC 3.6.1.17) [5], which cleaves A-5'-PPPP-5'A to yield AMP and ATP.
- A protein encoded on the antisense RNA of the basic fibroblast growth factor gene in higher vertebrates.
- Yeast protein YSA1.
- *Escherichia coli* hypothetical protein yfaO.
- *Escherichia coli* hypothetical protein ygdU and HI0901, the corresponding *Haemophilus influenzae* protein.
- *Escherichia coli* hypothetical protein yjaD and HI0432, the corresponding *Haemophilus influenzae* protein.
- *Escherichia coli* hypothetical protein yrfE.
- *Bacillus subtilis* hypothetical protein yqkG.
- *Bacillus subtilis* hypothetical protein yzgD.
- Yeast hypothetical protein YGL067w.

It is proposed [2] that the conserved domain could be involved in the active center of a family of pyrophosphate-releasing NTPases. As a signature pattern the core region of the domain was selected; it contains four conserved glutamate residues.

30 Consensus pattern: G-x(5)-E-x(4)-[STAGC]-[LIVMAC]-x-R-E-[LIVMFT]-x-E-E-

[1] Michaels M.L., Miller J.H. J. Bacteriol. 174:6321-6325(1992).

[2] Koonin E.V. Nucleic Acids Res. 21:4847-4847(1993).

- [3] Mejean V., Salles C., Bullions M.J., Bessman M.J., Claverys J.-P. *Mol. Microbiol.* 11:323-330(1994).
- [4] Sakumi K., Furuichi M., Tsuzuki T., Kakuma T., Kawabata S., Maki H., Sekiguchi M. J. *Biol. Chem.* 268:23524-23530(1993).
- 5 [5] Thorne N.M.H., Hankin S., Wilkinson M.C., Nunez C., Barraclough R., McLennan A.G. *Biochem. J.* 311:717-721(1995).

361. Myb DNA-binding domain repeat signatures

- The retroviral oncogene v-myb, and its cellular counterpart c-myb, encodenuclear DNA-binding proteins that specifically recognize the sequence YAAC(G/T)G [1]. The myb family also includes the following proteins: - *Drosophila* D-myb [2]. - Vertebrate myb-like proteins A-myb and B-myb [3]. - Maize C1 protein, a trans-acting factor which controls the expression of genes involved in anthocyanin biosynthesis. - Maize P protein [4], a trans-acting factor which regulates the biosynthetic pathway of a flavonoid-derived pigment in certain floral tissues. - *Arabidopsis thaliana* protein GL1 [5], required for the initiation of differentiation of leaf hair cells (trichomes). - A number of myb/c1-related proteins in maize and barley, whose roles are not yet known [4]. - Yeast BAS1 [7], a transcriptional activator for the HIS4 gene. - Yeast REB1 [8], which recognizes sites within both the enhancer and the promoter of rRNA transcription, as well as upstream of many genes transcribed by RNA polymerase II. - Fission yeast cdc5, a possible transcription factor whose activity is required for cell cycle progression and growth during G2. - Fission yeast myb1, which regulates telomere length and function. - Yeast hypothetical protein YMR213w. One of the most conserved regions in all of these proteins is a domain of 160 amino acids. It consists of three tandem repeats of 51 to 53 amino acids. In myb, this repeat region has been shown [9] to be involved in DNA-binding. The major part of the first repeat is missing in retroviral v-myb sequences and in plant myb-related proteins. Yeast REB1 differs from the other proteins in this family in having a single myb-like domain. As shown in the following schematic representation, two signature patterns for myb-like domains were developed; the first is located in the N-terminal section, the second spans the C-terminal extremity of the domain.
- 30 xxxxxxxxWxxxEDxxxxxxxxxxxxWxxIxxxxxxRxxxxxxxxWxxxx
 ***** : Position of the patterns.

Consensus pattern: W-[ST]-x(2)-E-[DE]-x(2)-[LIV]-

Consensus pattern: W-x(2)-[LI]-[SAG]-x(4,5)-R-x(8)-[YW]-x(3)-[LIVM]-

Note: this pattern detects the three copies of the domain in myb, d-myb, A-myb and B-myb; the second of the two complete copies of plant myb-related proteins, and the last two copies of yeast BAS1

- 5 [1] Biednkapp H., Borgmeyer U., Sippel A.E., Klempnauer K.-H. Nature 335:835-837(1988).
- [2] Peters C.W.B., Sippel A.E., Vingron M., Klempnauer K.-H. EMBO J. 6:3085-3090(1987).
- [3] Nomura N., Takahashi M., Matsui M., Ishii S., Date T., Sasamoto S., Ishizaki R. Nucleic
- 10 Acids Res. 16:11075-11090(1988).
- [4] Grotewold E., Athma P., Peterson T. Proc. Natl. Acad. Sci. U.S.A. 88:4587-4591(1991).
- [5] Oppenheimer D.G., Herman P.L., Sivakumaran S., Esch J., Marks M.D. Cell 67:483-493(1991).
- [6] Marocco A., Wissenbach M., Becker D., Paz-Ares J., Saedler H., Salamini F., Rohde W.
- 15 Mol. Gen. Genet. 216:183-187(1989).
- [7] Tice-Baldwin K., Fink G.R., Arndt K.T. Science 246:931-935(1989).
- [8] Ju Q., Morrow B.E., Warner J.R. Mol. Cell. Biol. 10:5226-5234(1990).
- [9] Klempnauer K.-H., Sippel A.E. EMBO J. 6:2719-2725(1987).

- 20 362. NAD-dependent glycerol-3-phosphate dehydrogenase signature
- NAD-dependent glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) (GPD) catalyzes the reversible reduction of dihydroxyacetone phosphate to glycerol-3- phosphate. It is a eukaryotic cytosolic homodimeric protein of about 40 Kd. As a signature pattern a glycine-rich region that is probably [1] involved in NAD-binding was selected.
- 25

Consensus pattern: G-[AT]-[LIVM]-K-[DN]-[LIVM](2)-A-x-[GA]-x-G-[LIVMF]-x-[DE]-G-[LIVM]-x-[LIVMFYW]-G-x-N-

- 30 [1] Otto J., Argos P., Rossmann M.G. Eur. J. Biochem. 109:325-330(1980).

363. Nucleosome assembly protein (NAP)

It is thought that NAPs may be involved in regulating gene expression as a result of histone accessibility [1].

[1] Rodriguez P, Munroe D, Prawitt D, Chu LL, Bric E, Kim J, Reid LH, Davies C, Nakagama H, Loebbert R, Winterpacht A, Petruzzi MJ, Higgins MJ, Nowak N, Evans G,

- 5 Shows T, Weissman BE, Zabel B, Housman DE, Pelletier J, Genomics 1997;44:253-265. [2] Schnieders F, Dork T, Arnemann J, Vogel T, Werner M, Schmidtke J; Hum Mol Genet 1996;5:1801-1807.

10 364. NB-ARC domain

van der Biezen EA, Jones JD, Curr Biol 1998;8:226-227.

365. Nucleoside diphosphate kinases active site

- 15 Nucleoside diphosphate kinases (EC 2.7.4.6) (NDK) [1] are enzymes required for the synthesis of nucleoside triphosphates (NTP) other than ATP. They provide NTPs for nucleic acid synthesis, CTP for lipid synthesis, UTP for polysaccharide synthesis and GTP for protein elongation, signal transduction and microtubule polymerization. In eukaryotes, there seems to be a small family of NDK isozymes each of which acts in a different subcellular
- 20 compartment and/or has a distinct biological function. Eukaryotic NDK isozymes are hexamers of two highly related chains (A and B) [2]. By random association (A6, A5B...AB5, B6), these two kinds of chain form isoenzymes differing in their isoelectric point. NDK are proteins of 17 Kd that act via a ping-pong mechanism in which a histidine residue is phosphorylated, by transfer of the terminal phosphate group from ATP. In the presence of
- 25 magnesium, the phosphoenzyme can transfer its phosphate group to any NDP, to produce an NTP. NDK isozymes have been sequenced from prokaryotic and eukaryotic sources. It has also been shown [3] that the *Drosophila* awd (abnormal wing discs) protein, is a microtubule-associated NDK. Mammalian NDK is also known as metastasis inhibition factor nm23. The sequence of NDK has been highly conserved through evolution. There is a single histidine
- 30 residue conserved in all known NDK isozymes, which is involved in the catalytic mechanism [2]. Our signature pattern contains this residue.

Consensus pattern: N-x(2)-H-[GA]-S-D-[SA]-[LIVMPKNE] [H is the putative active site residue]-

- [1] Parks R., Agarwal R. (In) The Enzymes (3rd edition) 8:307-334(1973).
[2] Gilles A.-M., Presecan E., Vonica A., Lascu I. J. Biol. Chem. 266:8784-8789(1991).
[3] Biggs J., Hersperger E., Steeg P.S., Liotta L.A., Shearn A. Cell 63:933-940(1990).

5

366. Nitrite and sulfite reductases iron-sulfur/siroheme-binding site (NIR_SIR)

Nitrite reductases (NiR) [1] catalyze the reduction of nitrite into ammonium, the second step in the assimilation of nitrate. There are two types of NiR: the higher plant chloroplastic form of NiR (EC 1.7.7.1) is a monomeric protein that uses reduced ferredoxin as the electron donor; while fungal and bacterial NiR (EC 1.6.6.4) are homodimeric proteins that uses NAD(P)H as the electron donor. Both forms of NiR contain a siroheme-Fe and iron-sulfur centers. Sulfite reductase (NADPH) (EC 1.8.1.2) (SIR) [2] is the bacterial enzyme that catalyzes the reduction of sulfite to sulfide. SIR is an oligomeric enzyme with a subunit composition of alpha(8)-beta(4), the alpha component is a flavoprotein (SIR-FP), while the beta component is a siroheme, iron-sulfurprotein (SIR-HP). Sulfite reductase (ferredoxin) (EC 1.8.7.1) [3] is a cyanobacterial and plant monomeric enzyme that also catalyzes the reduction of sulfite to sulfide. Anaerobic sulfite reductase (EC 1.8.1.-) (ASR) [4], a bacterial enzyme that catalyzes the NADH-dependent reduction of sulfite to sulfide. ASR is an oligomeric enzyme composed of three different subunits. The C component (geneasrC) seems to be a siroheme, iron-sulfur protein. These enzymes share a region of sequence similarity in their C-terminal half; this region which spans about 80 amino acids includes four conserved cysteine residues. Two of the Cys are grouped together at the beginning of the domain, and the two others are grouped in the middle of the domain. The cysteines are involved in the binding of the iron-sulfur center; the last one also binds the siroheme group [2]. A signature pattern from the region around the second cluster of cysteines was derived.

Consensus pattern: [STV]-G-C-x(3)-C-x(6)-[DE]-[LIVMF]-[GAT]-[LIVMF] [The two C's are iron-sulfur ligands]-

30

- [1] Campbell W.H., Kinghorn J.R. Trends Biochem. Sci. 15:315-319(1990).
[2] Crane B.R., Siegel L.M., Getzoff E.D. Science 270:59-67(1995).
[3] Gisselmann G., Klausmeier P., Schwenn J.D. Biochim. Biophys. Acta 1144:102-106(1993).

[4] Huang C.J., Barrett E.L. J. Bacteriol. 173:1544-1553(1991).

367. (NMT) Myristoyl-CoA:protein N-myristoyltransferase signatures. Myristoyl-CoA:
 5 protein N-myristoyltransferase (EC 2.3.1.97) (Nmt) [1] is the enzyme responsible for
 transferring a myristate group on the N-terminal glycine of a number of cellular eukaryotic
 and viral proteins. Nmt is a monomeric protein of about 50 to 60 Kd whose sequence appears
 to be well conserved. Two highly conserved regions have been developed as signature
 patterns. The first one is located in the central section, the second in the C-terminal part.

10 Consensus pattern: E-I-N-F-L-C-x-H-K-

Consensus pattern: K-F-G-x-G-D-G-

[1] Rudnick D.A., McWherter C.A., Gokel G.W., Gordon J.I. Adv. Enzymol. 67:375-
 15 430(1993).

368. ADP-glucose pyrophosphorylase signatures (NTP_transferase)

ADP-glucose pyrophosphorylase (glucose-1-phosphate adenyltransferase) [1,2](EC
 20 2.7.7.27) catalyzes a very important step in the biosynthesis of alpha 1,4-glucans (glycogen
 or starch) in bacteria and plants: synthesis of the activated glucosyl donor, ADP-glucose,
 from glucose-1-phosphate and ATP. ADP-glucose pyrophosphorylase is a tetrameric
 allosterically regulated enzyme. It is a homotetramer in bacteria while in plant chloroplasts
 and amyloplasts, it is a heterotetramer of two different, yet evolutionary related, subunits.

25 There are a number of conserved regions in the sequence of bacterial and plant ADP-glucose
 pyrophosphorylase subunits. Three of these regions were selected as signature patterns. The
 first two are N-terminal and have been proposed to be part of the allosteric and/or substrate-
 binding sites in the Escherichia coli enzyme (gene glgC). The third pattern corresponds to a
 conserved region in the central part of the enzymes.

30 Consensus pattern: [AG]-G-G-x-G-[STK]-x-L-x(2)-L-[TA]-x(3)-A-x-P-A-[LV] -

Consensus pattern: W-[FY]-x-G-[ST]-A-[DNSH]-[AS]-[LIVMFYW]-

Consensus pattern: [APV]-[GS]-M-G-[LIVMN]-Y-[IVC]-[LIVMFY]-x(2)-[DENPHK] -

[1] Nakata P.A., Greene T.W., Anderson J.M., Smith-White B.J., Okita T.W., Preiss J. Plant Mol. Biol. 17:1089-1093(1991).

[2] Preiss J., Ball K., Hutney J., Smith-White B.J., Li. L., Okitsa T.W. Pure Appl. Chem. 63:535-544(1991).

5

369. Sodium/hydrogen exchanger family

Na/H antiporters are key transporters in maintaining the pH of actively metabolizing cells. The molecular mechanisms of antiport are unclear.

10

These antiporters contain 10-12 transmembrane regions (M) at the amino-terminus and a large cytoplasmic region at the carboxyl terminus. The transmembrane regions M3-M12 share identity with other members of the family. The M6 and M7 regions are highly conserved. Thus, this is thought to be the region that is involved in the transport of sodium and hydrogen ions. The cytoplasmic region has little similarity throughout the family.

15

[1] Dibrov P, Fliegel L; FEBS Lett 1998;424:1-5. [2] Orlowski J, Grinstein S; J Biol Chem 1997;272:22373-22376.[3] Numata M, Petrecca K, Lake N, Orlowski J; J Biol Chem 1998;273:6951-6959.

20

370. Sodium:sulfate symporter family signature (Na_sulph_symp)

Integral membrane proteins that mediate the intake of a wide variety of molecules with the concomitant uptake of sodium ions (sodium symporters) can be grouped, on the basis of sequence and functional similarities into a number of distinct families. One of these families currently consists of the following proteins: - Mammalian sodium/sulfate cotransporter [1]. - Mammalian renal sodium/dicarboxylate cotransporter [2], which transports succinate and citrate. - Mammalian intestinal sodium/dicarboxylate cotransporter. - Chlamydomonas reinhardtii putative sulfur deprivation response regulator SAC1 [3]. - Caenorhabditis elegans hypothetical proteins B0285.6, F31F6.6, K08E5.2 and R107.1. - Escherichia coli hypothetical protein yfbs. - Haemophilus influenzae hypothetical protein HI0608. - Synechocystis strain PCC 6803 hypothetical protein sl10640. - Methanococcus jannaschii hypothetical protein MJ0672. These transporters are proteins of from 430 to 620 amino acids which are highly

30

hydrophobic and which probably contain about 12 transmembrane regions. As a signature pattern, a conserved region was selected which is located in or near the penultimate transmembrane region.

5 Consensus pattern: [STACP]-S-x(2)-F-x(2)-P-[LIVM]-[GSA]-x(3)-N-x-[LIVM]-V-

[1] Markovich D., Forgo J., Stange G., Biber J., Murer H. Proc. Natl. Acad. Sci. U.S.A. 90:8073-8077(1993).

[2] Pajor A.M. Am. J. Physiol. 270:642-648(1996).

10 [3] Davies J.P., Yildiz F.H., Grossman A. EMBO J. 15:2150-2159(1996).

371. NifU-like domain

This is an alignment of the carboxy-terminal domain. This is the only common region
15 between the NifU protein from nitrogen-fixing bacteria and rhodobacterial species. The biochemical function of NifU is unknown [1].

Ouzounis C, Bork P, Sander C, Trends Biochem Sci 1994;19:199-200.

20 372. Nitrilases / cyanide hydratase signatures

Nitrilases (EC 3.5.5.1) are enzymes that convert nitriles into their corresponding acids and ammonia. They are widespread in microbes as well as in plants where they convert indole-3-acetonitrile to the hormone indole-3-acetic acid. A conserved cysteine has been shown [1,2] to be essential for enzyme activity; it seems to be involved in a nucleophilic attack on the
25 nitrile carbon atom. Cyanide hydratase (EC 4.2.1.66) converts HCN to formamide. In phytopathogenic fungi, it is used to avoid the toxic effect of cyanide released by wounded plants [3]. The sequence of cyanide hydrolase is evolutionary related to that of nitrilases. Yeast hypothetical proteins YIL164c and YIL165c also belong to this family. As signature patterns for these enzymes, two conserved regions were selected. The first is located in the N-
30 terminal section while the second, which contains the active site cysteine, is located in the central section.

Consensus pattern: G-x(2)-[LIVMFY](2)-x-[IF]-x-E-x(2)-[LIVM]-x-G-Y-P-

Consensus pattern: G-[GAQ]-x(2)-C-[WA]-E-[NH]-x(2)-[PST]-[LIVMFYS]-x-[KR] [C is the active site residue]-

[1] Kobayashi M., Izui H., Nagasawa T., Yamada H. Proc. Natl. Acad. Sci. U.S.A. 90:247-251(1993).

[2] Kobayashi M., Komeda H., Yanaka N., Nagasawa T., Yamada H. J. Biol. Chem. 267:20746-20751(1992).

[3] Wang P., Vanetten H.D. Biochem. Biophys. Res. Commun. 187:1048-1054(1992).

373. NusB family

The NusB protein is involved in the regulation of rRNA biosynthesis by transcriptional antitermination.

Huenges M, Rolz C, Gschwind R, Peteranderl R, Berglechner F, Richter G, Bacher A, Kessler H, Gemmecker G, EMBO J 1998;17:4092-4100.

374. (Neur Chan) Neurotransmitter-gated ion-channels signature

Neurotransmitter-gated ion-channels [1,2,3,4] provide the molecular basis for rapid signal transmission at chemical synapses. They are post-synaptic oligomeric transmembrane complexes that transiently form a ionic channel upon the binding of a specific neurotransmitter. Presently, the sequence of subunits from five types of neurotransmitter-gated receptors are known: - The nicotinic acetylcholine receptor (AChR), an excitatory cation channel. In the motor endplates of vertebrates, it is composed of four different subunits (alpha, beta, gamma and delta or epsilon) with a molar stoichiometry of 2:1:1:1. In neurones, the AChR receptor is composed of two different types of subunits: alpha and non-alpha (also called beta). Nicotinic AChRs are also found in invertebrates. - The glycine receptor, an inhibitory chloride ion channel. The glycine receptor is a pentamer composed of two different subunits (alpha and beta). - The gamma-aminobutyric-acid (GABA) receptor, which is also an inhibitory chloride ion channel. The quaternary structure of the GABA receptor is complex; at least four classes of subunits are known to exist (alpha, beta, gamma, and delta) and there are many variants in each class (for example: six variants of the alpha class have already been sequenced). - The serotonin 5HT3 receptor. Serotonin is a biogenic hormone that functions as a neurotransmitter, a hormone and a mitogen. There are seven major groups

of serotonin receptors; six of these groups (5HT1, 5HT2, and 5HT4 to 5HT7) transduce extracellular signal by activating G proteins, while 5HT3 is a ligand-gated cation-specific ion channel which, when activated causes fast, depolarizing responses in neurons. - The glutamate receptor, an excitatory cation channel. Glutamate is the main excitatory

neurotransmitter in the brain. At least three different types of glutamate receptors have been described and are named according to their selective agonists (kainate, N-methyl-D-aspartate (NMDA) and quisqualate). All known sequences of subunits from neurotransmitter-gated ion-channels are structurally related. They are composed of a large extracellular glycosylated N-terminal ligand-binding domain, followed by three hydrophobic transmembrane regions which form the ionic channel, followed by an intracellular region of variable length. A fourth hydrophobic region is found at the C-terminal of the sequence. The sequence of subunits from the AchR, GABA, 5HT3, and Gly receptors are clearly evolutionary related and share many regions of sequence similarities. These sequence similarities are either absent or very weak in the Glu receptors. In the N-terminal extracellular domain of AchR/GABA/5HT3/Gly receptors, there are two conserved cysteine residues, which, in AchR, have been shown to form a disulfide bond essential to the tertiary structure of the receptor. A number of amino acids between the two disulfide-bonded cysteines are also conserved. Therefore this region was used as a signature pattern for this subclass of proteins.

Consensus pattern: C-x-[LIVMFQ]-x-[LIVMF]-x(2)-[FY]-P-x-D-x(3)-C [The two C's are linked by a disulfide bond]-

[1] Stroud R.M., McCarthy M.P., Shuster M. Biochemistry 29:11009-11023(1990).

[2] Betz H. Neuron 5:383-392(1990).

[3] Dingleline R., Myers S.J., Nicholas R.A. FASEB J. 4:2632-2645(1990).

[4] Barnard E.A. Trends Biochem. Sci. 17:368-374(1992).

375. Orotidine 5'-phosphate decarboxylase active site

Orotidine 5'-phosphate decarboxylase (EC 4.1.1.23) (OMPdecase) [1,2] catalyzes the last step in the de novo biosynthesis of pyrimidines, the decarboxylation of OMP into UMP. In higher eukaryotes OMPdecase is part, with orotatephosphoribosyltransferase, of a bifunctional enzyme, while the prokaryotic and fungal OMPdecases are monofunctional protein. Some parts of the sequence of OMPdecase are well conserved across species. The best conserved

region is located in the N-terminal half of OMPdecases and is centered around a lysine residue which is essential for the catalytic function of the enzyme. This region has been developed as a signature pattern.

- 5 Consensus pattern: [LIVMFTA]-[LIVMF]-x-D-x-K-x(2)-D-I-[GP]-x-T-[LIVMTA] [K is the active site residue]-

[1] Jacquet M., Guilbaud R., Garreau H. Mol. Gen. Genet. 211:441-445(1988).

[2] Kimsey H.H., Kaiser D. J. Biol. Chem. 267:819-824(1992).

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376. ATP synthase delta (OSCP) subunit signature

ATP synthase (proton-translocating ATPase) (EC 3.6.1.34) [1,2] is a component of the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), which acts as a proton channel, and a catalytic core, termed coupling factor CF(1).

One of the subunits of the ATPase complex, known as subunit delta in bacteria and chloroplasts or the Oligomycin Sensitivity Conferral Protein (OSCP) in mitochondria, seems to be part of the stalk that links CF(0) to CF(1). It either transmits conformational changes from CF(0) into CF(1) or is involved in proton conduction [3].

The different delta/OSCP subunits are proteins of approximately 200 amino-acid residues - once the transit peptide has been removed in the chloroplast and mitochondrial forms - which show only moderate sequence homology.

The signature pattern used to detect ATPase delta/OSCP subunits is based on a conserved region in the C-terminal section of these proteins.

Consensus pattern: [LIVM]-x-[LIVMFYT]-x(3)-[LIVMT]-[DENQK]-x(2)-[LIVM]-x-[GSA]-G-[LIVMFYGA]-x-[LIVM]-[KRHENQ]-x-[GSEN]

[1] Futai M., Noumi T., Maeda M. Annu. Rev. Biochem. 58:111-136(1989).

[2] Senior A.E. Physiol. Rev. 68:177-231(1988).

[3] Engelbrecht S., Junge W. Biochim. Biophys. Acta 1015:379-390(1990).

377. Aspartate and ornithine carbamoyltransferases signature

Aspartate carbamoyltransferase (EC 2.1.3.2) (ATCase) catalyzes the conversion of aspartate and carbamoyl phosphate to carbamoylaspartate, the second step in the *de novo* biosynthesis of pyrimidine nucleotides [1]. In prokaryotes ATCase consists of two subunits: a catalytic chain (gene *pyrB*) and a regulatory chain (gene *pyrI*), while in eukaryotes it is a domain in a multifunctional enzyme (called URA2 in yeast, rudimentary in *Drosophila*, and CAD in mammals [2]) that also catalyzes other steps of the biosynthesis of pyrimidines.

Ornithine carbamoyltransferase (EC 2.1.3.3) (OTCase) catalyzes the conversion of ornithine and carbamoyl phosphate to citrulline. In mammals this enzyme participates in the urea cycle [3] and is located in the mitochondrial matrix. In prokaryotes and eukaryotic microorganisms it is involved in the biosynthesis of arginine. In some bacterial species it is also involved in the degradation of arginine [4] (the arginine deaminase pathway).

It has been shown [5] that these two enzymes are evolutionary related. The predicted secondary structure of both enzymes are similar and there are some regions of sequence similarities. One of these regions includes three residues which have been shown, by crystallographic studies [6], to be implicated in binding the phosphoryl group of carbamoyl phosphate.

This region was selected as a signature for these enzymes.

Consensus pattern: F-x-[EK]-x-S-[GT]-R-T[S, R, and the 2nd T bind carbamoyl phosphate]

-Note: the residue in position 3 of the pattern allows to distinguish between an ATCase (Glu) and an OTCase (Lys).

[1] Lerner C.G., Switzer R.L. J. Biol. Chem. 261:11156-11165(1986).

[2] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B. BioEssays 15:157-164(1993).

[3] Takiguchi M., Matsubasa T., Amaya Y., Mori M. BioEssays 10:163-166(1989).

[4] Baur H., Stalon V., Falmagne P., Luethi E., Haas D. Eur. J. Biochem. 166:111-117(1987).

[5] Houghton J.E., Bencini D.A., O'Donovan G.A., Wild J.R. Proc. Natl. Acad. Sci. U.S.A. 81:4864-4868(1981).

[6] Ke H.-M., Honzatko R.B., Lipscomb W.N. Proc. Natl. Acad. Sci. U.S.A. 81:4037-4040(1984).

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378. Oleosins signature

Oleosins [1] are the proteinaceous components of plants' lipid storage bodies called oil bodies. Oil bodies are small droplets (0.2 to 1.5 μ m in diameter)

10 containing mostly triacylglycerol that are surrounded by a phospholipid/oleosin annulus. Oleosins may have a structural role in stabilizing the lipid body during desiccation of the seed, by preventing coalescence of the oil. They may also provide recognition signals for specific lipase anchorage in lipolysis during seedling growth. Oleosins are found in the monolayer lipid/

15 water interface of oil bodies and probably interact with both the lipid and phospholipid moieties.

Oleosins are proteins of 16 Kd to 24 Kd and are composed of three domains: an N-terminal hydrophilic region of variable length (from 30 to 60 residues); a central hydrophobic domain of about 70 residues and a C-terminal amphipathic

20 region of variable length (from 60 to 100 residues). The central hydrophobic domain is proposed to be made up of beta-strand structure and to interact with the lipids [2]. It is the only domain whose sequence is conserved and therefore a section from that domain was selected as a signature pattern.

25 Consensus pattern: [AG]-[ST]-x(2)-[AG]-x(2)-[LIVM]-[SAD]-T-P-[LIVMF](4)-F-S-P-[LIVM](3)-P-A

[1] Murphy D.J., Keen J.N., O'Sullivan J.N., Au D.M.Y., Edwards E.-W., Jackson P.J., Cummins I., Gibbons T., Shaw C.H., Ryan A.J. Biochim. Biophys. Acta 1088:86-94(1991).

30 [2] Tzen J.T.C., Lie G.C., Huang A.H.C. J. Biol. Chem. 267:15626-15634(1992).

379. (Orbi VP5) Orbivirus outer capsid protein VP5

This paper shows the location of the different capsid proteins and their relation to each other.

[1] Schoehn G, Moss SR, Nuttall PA, Hewat EA; Virology 1997;235:191-200.

5

380. Orn/DAP/Arg decarboxylases family 2 signatures

Pyridoxal-dependent decarboxylases acting on ornithine, lysine, arginine and related substrates can be classified into two different families on the basis of sequence similarities [1,2,3]. The second family consists of:

10

- Eukaryotic ornithine decarboxylase (EC 4.1.1.17) (ODC). ODC catalyzes the transformation of ornithine into putrescine.
- Prokaryotic diaminopimelic acid decarboxylase (EC 4.1.1.20) (DAPDC). DAPDC catalyzes the conversion of diaminopimelic acid into lysine; the last step in the biosynthesis of lysine.
- *Pseudomonas syringae* pv. *tabaci* protein *tabA*. *tabA* is probably involved in the biosynthesis of tabtoxin and is highly similar to DAPDC.
- Bacterial and plant biosynthetic arginine decarboxylase (EC 4.1.1.19) (ADC). ADC catalyzes the transformation of arginine into agmatine, the first step in the biosynthesis of putrescine from arginine.

15

20

The above proteins, while most probably evolutionary related, do not share extensive regions of sequence similarities. Two of the conserved regions were selected as signature patterns. The first pattern contains a conserved lysine residue which is known, in mouse ODC [4], to be the site of attachment of the pyridoxal-phosphate group. The second pattern contains a stretch of three consecutive glycine residues and has been proposed to be part of a substrate-binding region [5].

25

These enzymes are collectively known as group IV decarboxylases [3].

30

Consensus pattern: [FY]-[PA]-x-K-[SACV]-[NHCLFW]-x(4)-[LIVMF]-[LIVMTA]-x(2)-[LIVMA]-x(3)-[GTE] [K is the pyridoxal-P attachment site]
Consensus pattern: [GS]-x(2,6)-[LIVMSCP]-x(2)-[LIVMF]-[DNS]-[LIVMCA]-G-G-G-[LIVMFY]-[GSTPCEQ]

- [1] Bairoch A. Unpublished observations (1993).
- [2] Martin C., Cami B., Yeh P., Stragier P., Parsot C., Patte J.-C. Mol. Biol. Evol. 5:549-559(1988).
- [3] Sandmeier E., Hale T.I., Christen P. Eur. J. Biochem. 221:997-1002(1994).
- 5 [4] Poulin R., Lu L., Ackermann B., Bey P., Pegg A.E. J. Biol. Chem. 267:150-158(1992).
- [5] Moore R.C., Boyle S.M. J. Bacteriol. 172:4631-4640(1990).

381. Osteopontin signature

- 10 Osteopontin is an acidic phosphorylated glycoprotein of about 40 Kd which is abundant in the mineral matrix of bones and which binds tightly to hydroxyapatite [1,2,3]. It is suggested that osteopontin might function as a cell attachment factor and could play a key role in the adhesion of osteoclasts to the mineral matrix of bone.
- 15 Osteopontin-K is a kidney protein which is highly similar to osteopontin and probably also involved in cell-adhesion.
As a signature pattern a highly conserved region located at the N-terminal extremity of the mature protein was selected.
- 20 Consensus pattern: [KQ]-x-[TA]-x(2)-[GA]-S-S-E-E-K

- [1] Butler W.T. Connect. Tissue Res. 23:123-36(1989).
- [2] Gorski J.P. Calcif. Tissue Int. 50:391-396(1992).
- [3] Denhardt D.T., Guo X. FASEB J. 7:1475-1482(1993).

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382. Oxysterol-binding protein family signature

A number of eukaryotic proteins that seem to be involved with sterol synthesis and/or its regulation have been found [1] to be evolutionary related:

- 30 - Mammalian oxysterol-binding protein (OSBP). A protein of about 800 amino-acid residues that binds a variety of oxysterols: oxygenated derivatives of cholesterol. OSBP seems to play a complex role in the regulation of sterol metabolism.
- Yeast proteins HES1 and KES1; highly related proteins of 434 residues that

seem to play a role in ergosterol synthesis.

- Yeast OSH1, a protein of 859 residues that also plays a role in ergosterol synthesis. - Yeast hypothetical protein YHR001w (437 residues).

- Yeast hypothetical protein YHR073w (996 residues).

5 - Yeast hypothetical protein YKR003w (448 residues).

All these proteins contain a moderately conserved domain of about 250 residues located in the C-terminal half of OBSP, OSH1 and YHR073w and in the central section of the other proteins. As a signature pattern, the best conserved part was selected of this domain, a region that contains a conserved

10 pentapeptide.

Consensus pattern: E-[KQ]-x-S-H-[HR]-P-P-x-[STACF]-A

[1] Jiang B., Brown J.L., Sheraton J., Fortin N., Bussey H. Yeast 10:341-353(1994).

15

383. FMN oxidoreductase

20 384. Oxidoreductase FAD/NAD-binding domain

Number of members: 250

[1]

Medline: 92084635

The sequence of squash NADH:nitrate reductase and its

25 relationship to the sequences of other flavoprotein

oxidoreductases. A family of flavoprotein pyridine nucleotide cytochrome reductases.

Hyde GE, Crawford NM, Campbell W;

J Biol Chem 1991;266:23542-23547.

30 [2]Medline: 95111952

Crystal structure of the FAD-containing fragment of corn nitrate reductase at 2.5 Å resolution: relationship to other flavoprotein reductases.

Lu G, Campbell WH, Schneider G, Lindqvist Y;

Structure 1994;2:809-821.

385. (oxidored molyb) Eukaryotic molybdopterin oxidoreductases signature

5 A number of different eukaryotic oxidoreductases that require and bind a molybdopterin cofactor have been shown [1] to share a few regions of sequence similarity. These enzymes are:

- Xanthine dehydrogenase (EC 1.1.1.204), which catalyzes the oxidation of xanthine to uric acid with the concomitant reduction of NAD. Structurally,
10 this enzyme of about 1300 amino acids consists of at least three distinct domains: an N-terminal 2Fe-2S ferredoxin-like iron-sulfur binding domain (see <PDOC00175>), a central FAD/NAD-binding domain and a C-terminal Mo-
pterin domain.

- Aldehyde oxidase (EC 1.2.3.1), which catalyzes the oxidation aldehydes into
15 acids. Aldehyde oxidase is highly similar to xanthine dehydrogenase in its sequence and domain structure.

- Nitrate reductase (EC 1.6.6.1), which catalyzes the reduction of nitrate to nitrite. Structurally, this enzyme of about 900 amino acids consists of
an N-terminal Mo-pterin domain, a central cytochrome b5-type heme-binding
20 domain (see <PDOC00170>) and a C-terminal FAD/NAD-binding cytochrome reductase domain.

- Sulfite oxidase (EC 1.8.3.1), which catalyzes the oxidation of sulfite to sulfate. Structurally, this enzyme of about 460 amino acids consists of an
N-terminal cytochrome b5-binding domain followed by a Mo-pterin domain.

25 There are a few conserved regions in the sequence of the molybdopterin-binding domain of these enzymes. The pattern used to detect these proteins is based on one of them. It contains a cysteine residue which could be involved in binding the molybdopterin cofactor.

30 Consensus pattern: [GA]-x(3)-[KRNQHT]-x(11,14)-[LIVMFYWS]-x(8)-[LIVMF]-x-C-x(2)-
[DEN]-R-x(2)-[DE]

[1] Wootton J.C., Nicolson R.E., Cock J.M., Walters D.E., Burke J.F., Doyle W.A., Bray R.C. *Biochim. Biophys. Acta* 1057:157-185(1991).

386. (Oxidored q1) NADH-Ubiquinone/plastoquinone (complex I), various chains

This family is part of complex I which catalyses the transfer of two electrons from NADH to ubiquinone in a reaction that is associated with proton translocation across the membrane. Number of members: 1824

[1]

Medline: 93110040

The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains. Walker JE; Q Rev Biophys 1992;25:253-324.

387. (oxidored q3) NADH-ubiquinone/plastoquinone oxidoreductase chain 6. 179 members.

388. (oxidored q5) NADH-ubiquinone oxidoreductase chain 4, amino terminus

[1] Walker JE ; Q Rev Biophys 1992;25:253-324.

389. (oxidored q6) Respiratory-chain NADH dehydrogenase 20 Kd subunit signature Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there is one with a molecular weight of 20 Kd (in mammals) [3], which is a component of the iron-sulfur (IP) fragment of the enzyme. It seems to bind a 4Fe-4S iron-sulfur cluster. The 20 Kd subunit has been found to be:

- Nuclear encoded, as a precursor form with a transit peptide in mammals, and in *Neurospora crassa*.
- Mitochondrial encoded in *Paramecium* (gene *psbG*).
- Chloroplast encoded in various higher plants (gene *ndhK* or *psbG*).

The 20 Kd subunit is highly similar to [4]:

- Synechocystis strain PCC 6803 proteins psbG1 and psbG2.
- Subunit B of Escherichia coli NADH-ubiquinone oxidoreductase (gene nuoB).
- Subunit NQO6 of Paracoccus denitrificans NADH-ubiquinone oxidoreductase.
- Subunit 7 of Escherichia coli formate hydrogenlyase (gene hycG).
- 5 - Subunit I of Escherichia coli hydrogenase-4 (gene hyl).

As as signature pattern a highly conserved region was selected, located in the central section of this subunit and which contains a conserved cysteine that is probably involved in the binding of the 4Fe-4S center.

- 10 Consensus pattern: [GN]-x-D-[KRST]-[LIVMF](2)-P-[IV]-D-[LIVMFYW](2)-x-P-x-C-P-[PT] [The C is a putative 4Fe-4S ligand]

[1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).

[2] Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).

[3] Arizmendi J.M., Runswick M.J., Skehel J.M., Walker J.E. FEBS Lett. 301:237-

- 15 242(1992).

[4] Weidner U., Geier S., Ptöck A., Friedrich T., Leif H., Weiss H. J. Mol. Biol. 233:109-122(1993).

- 20 390. p53 tumor antigen signature

The p53 tumor antigen [1 to 5, E1,E2] is a protein found in increased amounts in a wide variety of transformed cells. It is also detectable in many proliferating nontransformed cells, but it is undetectable or present at low levels in resting cells. It is frequently mutated or inactivated in many types

- 25 of cancer. p53 seems to act as a tumor suppressor in some, but probably not all, tumor types. p53 is probably involved in cell cycle regulation, and may be a trans-activator that acts to negatively regulate cellular division by controlling a set of genes required for this process.

p53 is a phosphoprotein of about 390 amino acids which can be subdivided into

- 30 four domains: a highly charged acidic region of about 75 to 80 residues, a hydrophobic proline-rich domain (position 80 to 150), a central region (from 150 to about 300), and a highly basic C-terminal region. The sequence of p53 is well conserved in vertebrate species: attempts to identify p53 in other eukaryotic phylum has so far been unsuccessful.

As a signature pattern for p53 a perfectly conserved stretch of 13 residues located in the central region of the protein was selected. This region, known as domain IV in [3], is involved (along with an adjacent region) in the binding of the large T antigen of SV40. In man this region is the focus of a variety of point mutations in cancerous tumors.

Consensus pattern: M-C-N-S-S-C-M-G-G-M-N-R-R

[1] Levine A.J., Momand J., Finlay C.A. Nature 351:453-456(1991).

[2] Levine A.J., Momand J. Biochim. Biophys. Acta 1032:119-136(1990).

[3] Soussi T., Caron De Fromentel C., May P. Oncogene 5:945-952(1990).

[4] Lane D.P., Benchimol S. Genes Dev. 4:1-8(1990).

[5] Ulrich S.J., Anderson C.W., Mercer W.E., Appella E. J. Biol. Chem. 267:15259-15262(1992).

391. (P5CR) Delta 1-pyrroline-5-carboxylate reductase signature

Delta 1-pyrroline-5-carboxylate reductase (P5CR) (EC 1.5.1.2) [1,2] is the enzyme that catalyzes the terminal step in the biosynthesis of proline from glutamate, the NAD(P) dependent oxidation of 1-pyrroline-5-carboxylate into proline.

The sequences of P5CR from eubacteria (gene proC), archaeobacteria and eukaryotes show only a moderate level of overall similarity. As a signature pattern, the best conserved region located in the C-terminal section of P5CR was selected.

Consensus pattern: [PALF]-x(2,3)-[LIV]-x(3)-[LIVM]-[STAC]-[STV]-x-[GAN]-G-x-T-x(2)-[AG]-[LIV]-x(2)-[LMF]-[DENQK]

[1] Delauney A.J., Verma D.P. Mol. Gen. Genet. 221:299-305(1990).

[2] Savioz A., Jeenes D.J., Kocher H.P., Haas D. Gene 86:107-111(1990).

392. Poly-adenylate binding protein, unique domain.

393. (PAL) Phenylalanine and histidine ammonia-lyases active site

Phenylalanine ammonia-lyase (EC 4.3.1.5) (PAL) is a key enzyme of plant and fungi phenylpropanoid metabolism which is involved in the biosynthesis of a wide variety of secondary metabolites such as flavanoids, furanocoumarin phytoalexins and cell wall components. These compounds have many important roles in plants during normal growth and in responses to environmental stress. PAL catalyzes the removal of an ammonia group from phenylalanine to form trans-cinnamate.

Histidine ammonia-lyase (EC 4.3.1.3) (histidase) catalyzes the first step in histidine degradation, the removal of an ammonia group from histidine to produce urocanic acid.

The two types of enzymes are functionally and structurally related [1]. They are the only enzymes which are known to have the modified amino acid dehydroalanine (DHA) in their active site. A serine residue has been shown [2,3,4] to be the precursor of this essential electrophilic moiety. The region around this active site residue is well conserved and can be used as a signature pattern.

Consensus pattern: G-[STG]-[LIVM]-[STG]-[AC]-S-G-[DH]-L-x-P-L-[SA]-x(2)-[SA] [S is the active site residue]

[1] Taylor R.G., Lambert M.A., Sexsmith E., Sadler S.J., Ray P.N., Mahuran D.J., McInnes R.R. J. Biol. Chem. 265:18192-18199(1990).

[2] Langer M., Reck G., Reed J., Retey J. Biochemistry 33:6462-6467(1994).

[3] Schuster B., Retey J. FEBS Lett. 349:252-254(1994).

[4] Taylor R.G., McInnes R.R. J. Biol. Chem. 269:27473-27477(1994).

394. PAS domain

!- CAUTION. This family does not currently match all known examples of PAS domains.

PAS motifs appear in archaea, eubacteria and eukarya. Probably the most surprising identification of a PAS domain was that in

EAG-like K⁺-channels[1,3].

Number of members: 308

[1]

Medline: 97446881

- 5 PAS domain S-boxes in archaea, bacteria and sensors for oxygen and redox.

Zhulin IB, Taylor BL, Dixon R;

Trends Biochem Sci 1997;22:331-333.

[2]Medline: 95275818

- 10 1.4 A structure of photoactive yellow protein, a cytosolic photoreceptor: unusual fold, active site, and chromophore.

Borgstahl GE, Williams DR, Getzoff ED;

Biochemistry 1995;34:6278-6287.

[3]Medline: 98044337

- 15 PAS: a multifunctional domain family comes to light.

Ponting CP, Aravind L;

Curr Biol 1997;7:674-677.

- 20 395. (PBP) Phosphatidylethanolamine-binding protein family signature
Mammalian phosphatidylethanolamine-binding protein (also known as basic cytosolic 21 Kd protein) is a 186 residue protein found in a variety of tissues [1]. It binds hydrophobic ligands, such as phosphatidylethanolamine, but also seems [2] to bind nucleotides such as GTP and FMN, it is suggested
25 that it could act in membrane remodeling during growth and maturation. This protein belongs to a family that also includes:
 - Drosophila antennal protein A5, a putative odorant-binding protein.
 - Onchocerca volvulus antigen Ov-16 and the related proteins D1, D2 and D3.
 - Plasmodium falciparum putative phosphatidylethanolamine-binding protein.
- 30 - Toxocara canis secreted antigen TES-26. This larval protein has been shown to bind phosphatidylethanolamine.
- Yeast protein DKA1 (also known as NSP1 or TFS1). The function of this protein is not very clear. - Yeast hypothetical protein YLR179C.
- Caenorhabditis elegans hypothetical protein F40A3.3.

As a signature pattern, the best conserved region was selected which is located in the end of the first third of the sequence of these proteins.

Consensus pattern: [FYL]-x-[LV]-[LIVF]-x-[TIV]-[DC]-P-D-x-P-[SN]-x(10)-H

- [1] Seddiqi N., Bollengier F., Alliel P.M., Perin J.P., Bonnet F., Bucquoy S., Jolles P., Schoentgen F. J. Mol. Evol. 39:655-660(1994).
[2] Schoentgen F., Jolles P. FEBS Lett. 369:22-6(1995).

396. PCI domain

This domain has also been called the PINT motif (Proteasome, Int-6, Nip-1 and TRIP-15) [1].

Number of members: 49

[1]

Medline: 98308842

The PCI domain: a common theme in three multiprotein complexes.

Hofmann K, Bucher P;

Trends Biochem Sci 1998;23:204-205.

[2]Medline: 98266368

Homologues of 26S proteasome subunits are regulators of transcription and translation.

Aravind L, Ponting CP;

Protein Sci 1998;7:1250-1254.

397. (PCMT) Protein-L-isoaspartate (D-aspartate) O-methyltransferase signature. Protein-L-isoaspartate (D-aspartate) O-methyltransferase (EC 2.1.1.77) (PCMT)[1] (which is also known as L-isoaspartyl protein carboxyl methyltransferase) is an enzyme that catalyzes the transfer of a methyl group from S-adenosylmethionine to the free carboxyl groups of D-aspartyl or L-isoaspartyl residues in a variety of peptides and proteins. The enzyme does not act on normal L-aspartyl residues L-isoaspartyl and D-aspartyl are the products of the spontaneous deamidation and/or isomerization of normal L-aspartyl and L-asparaginy

residues in proteins. PCMT plays a role in the repair and/or degradation of these damaged proteins; the enzymatic methyl esterification of the abnormal residues can lead to their conversion to normal L-aspartyl residues. PCMT is a well-conserved and widely distributed cytosolic protein of about 24Kd. As a signature pattern, a conserved region in the central part of this enzyme has been developed.

Consensus pattern: [GSA]-D-G-x(2)-G-[FYWV]-x(3)-[AS]-P-[FY]-[DN]-x-I-

[1] Kagan R.M., McFadden H.J., McFadden P.N., O'Connor C., Clarke S. Comp. Biochem. Physiol. 117b:379-385(1997).

398. (PCNA) Proliferating cell nuclear antigen signatures

Proliferating cell nuclear antigen (PCNA) [1,2] is a protein involved in DNA replication by acting as a cofactor for DNA polymerase delta, the polymerase responsible for leading strand DNA replication.

A similar protein exists in yeast (gene POL30) [3] and is associated with polymerase III, the yeast analog of polymerase delta. In baculoviruses the ETL protein has been shown [4] to be highly related to PCNA and is probably associated with the viral encoded DNA polymerase. An homolog of PCNA is also found in archebacteria.

As signatures for this family of proteins, two conserved regions were selected located in the N-terminal section. The second one has been proposed to bind DNA.

Consensus pattern: [GA]-[LIVMF]-x-[LIVMA]-x-[SAV]-[LIVM]-D-x-[NSAE]-[HKR]-[VI]-x-[LY]-[VGA]-x-[LIVM]-x-[LIVM]-x(4)-F

-Consensus pattern: [RKA]-C-[DE]-[RH]-x(3)-[LIVMF]-x(3)-[LIVM]-x-[SGAN]-[LIVMF]-x-K-[LIVMF](2)

[1] Bravo R., Frank R., Blundell P.A., McDonald-Bravo H. Nature 326:515-517(1987).

[2] Suzuka I., Hata S., Matsuoka M., Kosugi S., Hashimoto J. Eur. J. Biochem. 195:571-575(1991). [3] Bauer G.A., Burgess P.M.J. Nucleic Acids Res. 18:261-265(1990).

[4] O'Reilly D.R., Crawford A.M., Miller L.K. Nature 337:606-606(1989).

399. (PDT) Prephenate dehydratase signatures

Prephenate dehydratase (EC 4.2.1.51) (PDT) catalyzes the decarboxylation of prephenate into phenylpyruvate. In microorganisms PDT is involved in the terminal pathway of the biosynthesis of phenylalanine. In some bacteria such as *Escherichia coli* PDT is part of a bifunctional enzyme (P-protein) that also catalyzes the transformation of chorismate into prephenate (chorismate mutase) while in other bacteria it is a monofunctional enzyme. The sequence of monofunctional PDT align well with the C-terminal part of that of P-proteins [1].

As signature patterns for PDT two conserved regions were selected. The first region contains a conserved threonine which has been said to be essential for the activity of the enzyme in *E. coli*. The second region includes a conserved glutamate. Both regions are in the C-terminal part of PDT.

Consensus pattern: [FY]-x-[LIVM]-x(2)-[LIVM]-x(5)-[DN]-x(5)-T-R-F-[LIVMW]-x-[LIVM]

[1] Fischer R.S., Zhao G., Jensen R.A. *J. Gen. Microbiol.* 137:1293-1301(1991).

400. PDZ domain (Also known as DHR or GLGF).

PDZ domains are found in diverse signaling proteins.

[1] Ponting CP, Phillips C, Davies KE, Blake DJ

Bioessays 1997;19:469-479. [2] Doyle DA, Lee A, Lewis J, Kim E, Sheng M, MacKinnon R; *Cell*. 1996;85:1067-1076. [3] Ponting CP; *Protein Sci* 1997;6:464-468.

401. (PPDK_N_term) PEP-utilizing enzymes signatures

A number of enzymes that catalyze the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) via a phospho-histidine intermediate have been shown to be structurally related [1,2,3,4]. These enzymes are:

- Pyruvate, orthophosphate dikinase (EC 2.7.9.1) (PPDK). PPDK catalyzes the

reversible phosphorylation of pyruvate and phosphate by ATP to PEP and diphosphate. In plants PPK function in the direction of the formation of PEP, which is the primary acceptor of carbon dioxide in C4 and crassulacean acid metabolism plants. In some bacteria, such as *Bacteroides symbiosus*,

5 PPK functions in the direction of ATP synthesis.

- Phosphoenolpyruvate synthase (EC 2.7.9.2) (pyruvate, water dikinase). This enzyme catalyzes the reversible phosphorylation of pyruvate by ATP to form PEP, AMP and phosphate, an essential step in gluconeogenesis when pyruvate and lactate are used as a carbon source.

10 - Phosphoenolpyruvate-protein phosphotransferase (EC 2.7.3.9). This is the first enzyme of the phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS), a major carbohydrate transport system in bacteria. The PTS catalyzes the phosphorylation of incoming sugar substrates concomitant with their translocation across the cell membrane. The general mechanism
15 of the PTS is the following: a phosphoryl group from PEP is transferred to enzyme-I (EI) of PTS which in turn transfers it to a phosphoryl carrier protein (HPr). Phospho-HPr then transfers the phosphoryl group to a sugar-specific permease.

All these enzymes share the same catalytic mechanism: they bind PEP and
20 transfer the phosphoryl group from it to a histidine residue. The sequence around that residue is highly conserved and can be used as a signature pattern for these enzymes. As a second signature pattern a conserved region was selected in the C-terminal part of the PEP-utilizing enzymes. The biological significance of this region is not yet known.

25 Consensus pattern: G-[GA]-x-[TN]-x-H-[STA]-[STAV]-[LIVM](2)-[STAV]-[RG] [H is phosphorylated]

-Consensus pattern: [DEQSK]-x-[LIVMF]-S-[LIVMF]-G-[ST]-N-D-[LIVM]-x-Q-[LIVMFYGT]-[STALIV]-[LIVMF]-[GAS]-x(2)-R

30

[1] Reizer J., Hoischen C., Reizer A., Pham T.N., Saier M.H. Jr. Protein Sci. 2:506-521(1993).

[2] Reizer J., Reizer A., Merrick M.J., Plunkett G. III, Rose D.J., Saier M.H. Jr. Gene 181:103-108(1996).

[3] Pocalyko D.J., Carroll L.J., Martin B.M., Babbitt P.C., Dunaway-Mariano D. Biochemistry 29:10757-10765(1990).

[4] Niersbach M., Kreuzaler F., Geerse R.H., Postma P., Hirsch H.J. Mol. Gen. Genet. 232:332-336(1992).

5

402. (PEPCK ATP) Phosphoenolpyruvate carboxykinase (ATP) signature Phosphoenolpyruvate carboxykinase (ATP) (EC 4.1.1.49) (PEPCK) [1] catalyzes the formation of phosphoenolpyruvate by decarboxylation of oxaloacetate while hydrolyzing ATP, a rate limiting step in gluconeogenesis (the biosynthesis of glucose).

The sequence of this enzyme has been obtained from Escherichia coli, yeast, and Trypanosoma brucei; these three sequences are evolutionarily related and share many regions of similarity. As a signature pattern a highly conserved region was selected that contains four acidic residues and which is located in the central part of the enzyme. The beginning of the pattern is located about 10 residues to the C-terminus of an ATP-binding motif 'A' (P-loop) (see <PDOC00017>) and is also part of the ATP-binding domain [2].

Consensus pattern: L-I-G-D-D-E-H-x-W-x-[DE]-x-G-[IV]-x-N

-Note: phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) an enzyme that catalyzes the same reaction, but using GTP instead of ATP, is not related to the above enzyme (see <PDOC00421>).

[1] Medina V., Pontarollo R., Glaeske D., Tabel H., Goldie H. J. Bacteriol. 172:7151-7156(1990).

[2] Matte A., Goldie H., Sweet R.M., Delbaere L.T.J. J. Mol. Biol. 256:126-143(1996).

403. (Pepcase) Phosphoenolpyruvate carboxylase active sites. Phosphoenolpyruvate carboxylase (EC 4.1.1.31) (PEPcase) catalyzes the irreversible beta-carboxylation of phosphoenolpyruvate by bicarbonate to yield oxaloacetate and phosphate. The enzyme is found in all plants and in a variety of microorganisms. A histidine [1] and a lysine [2] have been implicated in the catalytic mechanism of this enzyme; the regions around these active

site residues are highly conserved in PEPcase from various plants, bacteria and cyanobacteria and can be used as a signature patterns for this type of enzyme.

Consensus pattern: [VT]-x-T-A-H-P-T-[EQ]-x(2)-R-[KRH] [H is an active site residue]-

- 5 Consensus pattern: [IV]-M-[LIVM]-G-Y-S-D-S-x-K-D-[STAG]-G [K is an active site residue]-

[1] Terada K., Izui K. Eur. J. Biochem. 202:797-803(1991).[2] Jiao J.-A., Podesta F.E., Chollet R., O'Leary M.H., Andreo C.S. Biochim. Biophys. Acta 1041:291-295(1990).

10

404. PET112 family signature

The following proteins from eukaryotes, prokaryotes and archaebacteria belong to the same family:

- 15 - Yeast mitochondrial protein PET112 [1], which plays an unknown role in the expression of mitochondrial genes, probably at the level of translation.
 - *Aspergillus nidulans* mitochondrial protein nempA.
 - *Bacillus subtilis* hypothetical protein yzdD.
 - *Moraxella catarrhalis* hypothetical protein in bloR-1 3'region.
 20 - *Mycoplasma genitalium* hypothetical protein MG100.
 - *Methanococcus jannaschii* hypothetical proteins MJ0019 and MJ0160.

The size of these proteins range from 419 to 630 amino acids. As a signature pattern, a conserved region located in the N-terminal section was selected.

- 25 Consensus pattern: [DN]-x-[DN]-R-x(3)-P-L-[LIV]-E-[LIV]-x-[ST]-x-P

[1] Mulero J.J., Rosenthal J.K., Fox T.D. Curr. Genet. 25:299-304(1994).

30 405. (PFK) Phosphofructokinase signature

Phosphofructokinase (EC 2.7.1.11) (PFK) [1,2] is a key regulatory enzyme in the glycolytic pathway. It catalyzes the phosphorylation by ATP of fructose 6-phosphate to fructose 1,6-bisphosphate. In bacteria PFK is a tetramer of identical 36 Kd subunits. In mammals it is a tetramer of 80 Kd subunits. Each

80 Kd subunit consist of two homologous domains which are highly related to the bacterial 36 Kd subunits. In Human there are three, tissue-specific, types of PFK isozymes: PFKM (muscle), PFKL (liver), and PFKP (platelet). In yeast PFK is an octamer composed of four 100 Kd alpha chains (gene PFK1) and four
5 100 Kd beta chains (gene PFK2); like the mammalian 80 Kd subunits, the yeast 100 Kd subunits are composed of two homologous domains. As a signature pattern for PFK a region that contains three basic residues involved in fructose-6-phosphate binding was selected.

10 Consensus pattern: [RK]-x(4)-G-H-x-Q-[QR]-G-G-x(5)-D-R [The R/K, the H and the Q/R are involved in fructose-6-P binding]

-Note: Escherichia coli has two phosphofructokinase isozymes which are encoded by genes pfkA (major) and pfkB (minor). The pfkB isozyme is not evolutionary related to other prokaryotic or eukaryotic PFK's (see <PDOC00504>).

15 [1] Poorman R.A., Randolph A., Kemp R.G., Heinrikson R.L. Nature 309:467-469(1984).
[2] Heinisch J., Ritzel R.G., von Borstel R.C., Aguilera A., Rodicio R., Zimmermann F.K. Gene 78:309-321(1989).

20 406. (PGAM) Phosphoglycerate mutase family phosphohistidine signature
Phosphoglycerate mutase (EC 5.4.2.1) (PGAM) and bisphosphoglycerate mutase (EC 5.4.2.4) (BPGM) are structurally related enzymes which catalyze reactions involving the transfer of phospho groups between the three carbon atoms of
25 phosphoglycerate [1,2]. Both enzymes can catalyze three different reactions, although in different proportions:

- The isomerization of 2-phosphoglycerate (2-PGA) to 3-phosphoglycerate (3-PGA) with 2,3-diphosphoglycerate (2,3-DPG) as the primer of the reaction.
- The synthesis of 2,3-DPG from 1,3-DPG with 3-PGA as a primer.
- 30 - The degradation of 2,3-DPG to 3-PGA (phosphatase EC 3.1.3.13 activity).

In mammals, PGAM is a dimeric protein. There are two isoforms of PGAM: the M (muscle) and B (brain) forms. In yeast, PGAM is a tetrameric protein. BPGM is a dimeric protein and is found mainly in erythrocytes where it plays a major role in regulating hemoglobin oxygen affinity as a consequence of controlling

2,3-DPG concentration.

The catalytic mechanism of both PGAM and BPGM involves the formation of a phosphohistidine intermediate [3].

The bifunctional enzyme 6-phosphofructo-2-kinase / fructose-2,6-bisphosphatase (EC 2.7.1.105 and EC 3.1.3.46) (PF2K) [4] catalyzes both the synthesis and the degradation of fructose-2,6-bisphosphate. PF2K is an important enzyme in the regulation of hepatic carbohydrate metabolism. Like PGAM/BPGM, the fructose-2,6-bisphosphatase reaction involves a phosphohistidine intermediate and the phosphatase domain of PF2K is structurally related to PGAM/BPGM.

The bacterial enzyme alpha-ribazole-5'-phosphate phosphatase (gene cobC) which is involved in cobalamin biosynthesis also belongs to this family [5].

A signature pattern was built around the phosphohistidine residue.

Consensus pattern: [LIVM]-x-R-H-G-[EQ]-x(3)-N [H is the phosphohistidine residue]

-Note: some organisms harbor a form of PGAM independent of 2,3-DPG, this enzyme is not related to the family described above [6].

[1] Le Boulch P., Joulin V., Garel M.-C., Rosa J., Cohen-Solal M. Biochem. Biophys. Res. Commun. 156:874-881(1988).

[2] White M.F., Fothergill-Gilmore L.A. FEBS Lett. 229:383-387(1988).

[3] Rose Z.B. Meth. Enzymol. 87:43-51(1982).

[4] Bazan J.F., Fletterick R.J., Pilgis S.J. Proc. Natl. Acad. Sci. U.S.A. 86:9642-9646(1989).

[5] O'Toole G.A., Trzebiatowski J.R., Escalante-Semerena J.C. J. Biol. Chem. 269:26503-26511(1994).

[6] Grana X., De Lecea L., El-Maghrabi M.R., Urena J.M., Caellas C., Carreras J., Puigdomenech P., Pilgis S.J., Climent F. J. Biol. Chem. 267:12797-12803(1992).

407. (PGI) Phosphoglucose isomerase signatures

Phosphoglucose isomerase (EC 5.3.1.9) (PGI) [1,2] is a dimeric enzyme that catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate. PGI is involved in different pathways: in most higher organisms it is involved in glycolysis; in mammals it is involved in gluconeogenesis; in

plants in carbohydrate biosynthesis; in some bacteria it provides a gateway for fructose into the Entner-Doudouroff pathway. PGI has been shown [3] to be identical to neuroleukin, a neurotrophic factor which supports the survival of various types of neurons.

- 5 The sequence of PGI from many species ranging from bacteria to mammals is available and has been shown to be highly conserved. As signature patterns for this enzyme two conserved regions were selected, the first region is located in the central section of PGI, while the second one is located in its C-terminal section.

10

Consensus pattern: [DENS]-x-[LIVM]-G-G-R-[FY]-S-[LIVMT]-x-[STA]-[PSAC]-[LIVMA]-G

-Consensus pattern: [GS]-x-[LIVM]-[LIVMFYW]-x(4)-[FY]-[DN]-Q-x-G-V-E-x(2)-K

15

[1] Achari A., Marshall S.E., Muirhewad H., Palmieri R.H., Noltmann E.A. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 293:145-157(1981).

[2] Smith M.W., Doolittle R.F. J. Mol. Evol. 34:544-545(1992).

[3] Faik P., Walker J.I.H., Redmill A.A.M., Morgan M.J. Nature 332:455-456(1988).

20

408. (PGK) Phosphoglycerate kinase signature

Phosphoglycerate kinase (EC 2.7.2.3) (PGK) [1] catalyzes the second step in the second phase of glycolysis, the reversible conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate with generation of one molecule of ATP. PGK is found in all living organisms and its sequence has been highly conserved throughout evolution. It is a two-domain protein; each domain is composed of six repeats of an alpha/beta structural motif. As a signature pattern for PGK's, a conserved region in the N-terminal region was selected.

25

Consensus pattern: [KRHGTVCVN]-[VT]-[LIVMF]-[LIVMC]-R-x-D-x-N-[SACV]-P

30

[1] Watson H.C., Littlechild J.A. Biochem. Soc. Trans. 18:187-190(1990).

409. (PGM PMM) Phosphoglucomutase and phosphomannomutase phosphoserine signature

- Phosphoglucumutase (EC 5.4.2.2) (PGM). PGM is an enzyme responsible for the conversion of D-glucose 1-phosphate into D-glucose 6-phosphate. PGM participates in both the breakdown and synthesis of glucose [1].
- 5 - Phosphomannomutase (EC 5.4.2.8) (PMM). PMM is an enzyme responsible for the conversion of D-mannose 1-phosphate into D-mannose 6-phosphate. PMM is required for different biosynthetic pathways in bacteria. For example, in enterobacteria such as *Escherichia coli* there are two different genes coding for this enzyme: *rfbK* which is involved in the synthesis of the O antigen of lipopolysaccharide and *cpsG* which is required for the synthesis
- 10 of the M antigen capsular polysaccharide [2]. In *Pseudomonas aeruginosa* PMM (gene *algC*) is involved in the biosynthesis of the alginate layer [3] and in *Xanthomonas campestris* (gene *xanA*) it is involved in the biosynthesis of xanthan [4]. In *Rhizobium* strain *ngr234* (gene *noeK*) it is involved in the biosynthesis of the nod factor.
- 15 - Phosphoacetylglucosamine mutase (EC 5.4.2.3) which converts N-acetyl-D-glucosamine 1-phosphate into the 6-phosphate isomer.
- The catalytic mechanism of both PGM and PMM involves the formation of a phosphoserine intermediate [1]. The sequence around the serine residue is well conserved and can be used as a signature pattern.
- 20 In addition to PGM and PMM there are at least three uncharacterized proteins that belong to this family [5,6]:
- Urease operon protein *ureC* from *Helicobacter pylori*.
 - *Escherichia coli* protein *mrsA*.
 - *Paramecium tetraurelia* *parafusin*, a phosphoglycoprotein involved in
 - 25 exocytosis.
 - A *Methanococcus vannielii* hypothetical protein in the 3' region of the gene for ribosomal protein S10.

Consensus pattern: [GSA]-[LIVM]-x-[LIVM]-[ST]-[PGA]-S-H-x-P-x(4)-[GNHE] [S is the

30 phosphoserine residue]

-Note: PMM from fungi do not belong to this family.

- [2] Stevenson G., Lee S.J., Romana L.K., Reeves P.R. Mol. Gen. Genet. 227:173-180(1991).
- [3] Zielinski N.A., Chakrabarty A.M., Berry A. J. Biol. Chem. 266:9754-9763(1991).
- [4] Koeplin R., Arnold W., Hoette B., Simon R., Wang G., Puehler A. J. Bacteriol. 174:191-199(1992).
- [5] Bairoch A. Unpublished observations (1993).
- [6] Subramanian S.V., Wyroba E., Andersen A.P., Satir B.H. Proc. Natl. Acad. Sci. U.S.A. 91:9832-9836(1994).

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410. PH domain profile

The 'pleckstrin homology' (PH) domain is a domain of about 100 residues that occurs in a wide range of proteins involved in intracellular signaling or as constituents of the cytoskeleton [1 to 7].

15

The function of this domain is not clear, several putative functions have been suggested: - binding to the beta/gamma subunit of heterotrimeric G proteins, - binding to lipids, e.g. phosphatidylinositol-4,5-bisphosphate, - binding to phosphorylated Ser/Thr residues, - attachment to membranes by an unknown mechanism.

20

It is possible that different PH domains have totally different ligand requirements.

The 3D structure of several PH domains has been determined [8]. All known cases have a common structure consisting of two perpendicular anti-parallel beta sheets, followed by a C-terminal amphipathic helix. The loops connecting the beta-strands differ greatly in length, making the PH domain relatively difficult to detect. There are no totally invariant residues within the PH domain.

25

Proteins reported to contain one more PH domains belong to the following families:

30

- Pleckstrin, the protein where this domain was first detected, is the major substrate of protein kinase C in platelets. Pleckstrin is one of the rare proteins to contain two PH domains.
- Ser/Thr protein kinases such as the Act/Rac family, the beta-adrenergic receptor kinases, the mu isoform of PKC and the trypanosomal NrkA family.

- Tyrosine protein kinases belonging to the Btk/Itk/Tec subfamily.
- Insulin Receptor Substrate 1 (IRS-1).
- Regulators of small G-proteins like guanine nucleotide releasing factor GNRP (Ras-GRF) (which contains 2 PH domains), guanine nucleotide exchange proteins like vav, dbI, SoS and yeast CDC24, GTPase activating proteins like rasGAP and BEM2/IPL2, and the human break point cluster protein bcr.
- Cytoskeletal proteins such as dynamin (see <PDOC00362>), Caenorhabditis elegans kinesin-like protein unc-104 (see <PDOC00343>), spectrin beta-chain, syntrophin (2 PH domains) and yeast nuclear migration protein NUM1.
- Mammalian phosphatidylinositol-specific phospholipase C (PI-PLC) (see <PDOC50007>) isoforms gamma and delta. Isoform gamma contains two PH domains, the second one is split into two parts separated by about 400 residues.
- Oxysterol binding proteins OSBP, yeast OSH1 and YHR073w.
- Mouse protein citron, a putative rho/rac effector that binds to the GTP-bound forms of rho and rac,
- Several yeast proteins involved in cell cycle regulation and bud formation like BEM2, BEM3, BUD4 and the BEM1-binding proteins BOI2 (BEB1) and BOI1 (BOB1).
- Caenorhabditis elegans protein MIG-10.
- Caenorhabditis elegans hypothetical proteins C04D8.1, K06H7.4 and ZK632.12.
- Yeast hypothetical proteins YBR129c and YHR155w.

The profile for the PH domain, which has been developed by Toby Gibson at the EMBL, covers the total length of domain. Several proteins contain large insertions in the PH domain and are thus difficult to detect with this profile. In some of these cases, the profile will align only to one half of the PH domain.

- Sequences known to belong to this class detected by the pattern: ALL. But it should be noted that while all sequences containing PH domains are detected, not all PH domains are. Some of the split domains lie below the cutoff threshold.

- [1] Mayer B.J., Ren R., Clark K.L., Baltimore D. Cell 73:629-630(1993).
- [2] Haslam R.J., Koide H.B., Hemmings B.A. Nature 363:309-310(1993).
- [3] Musacchio A., Gibson T.J., Rice P., Thompson J., Saraste M. Trends Biochem. Sci. 18:343-348(1993).
- [4] Gibson T.J., Hyvonen M., Musacchio A., Saraste M., Birney E.

- Trends Biochem. Sci. 19:349-353(1994). [5] Pawson T.
 Nature 373:573-580(1995). [6] Ingley E., Hemmings B.A.
 J. Cell. Biochem. 56:436-443(1994). [7] Saraste M., Hyvonen M.
 Curr. Opin. Struct. Biol. 5:403-408(1995). [8] Riddihough G.
 5 Nat. Struct. Biol. 1:755-757(1994).

411. PHD-finger

[1]

10 Medline: 95216093

The PHD finger: implications for chromatin-mediated
 transcriptional regulation.

Aasland R, Gibson TJ, Stewart AF;
 Trends Biochem Sci 1995;20:56-59.

15 Number of members: 181

412. (PI-PLC-X) Phosphatidylinositol-specific phospholipase C profiles
 Phosphatidylinositol-specific phospholipase C (EC 3.1.4.11), an eukaryotic
 20 intracellular enzyme, plays an important role in signal transduction processes
 [1]. It catalyzes the hydrolysis of 1-phosphatidyl-D-myo-inositol-3,4,5-
 triphosphate into the second messenger molecules diacylglycerol and inositol-
 1,4,5-triphosphate. This catalytic process is tightly regulated by reversible
 phosphorylation and binding of regulatory proteins [2 to 4].

25 In mammals, there are at least 6 different isoforms of PI-PLC, they differ in
 their domain structure, their regulation, and their tissue distribution. Lower
 eukaryotes also possess multiple isoforms of PI-PLC.

All eukaryotic PI-PLCs contain two regions of homology, sometimes referred to
 as 'X-box' and 'Y-box'. The order of these two regions is always the same
 30 (NH2-X-Y-COOH), but the spacing is variable. In most isoforms, the distance
 between these two regions is only 50-100 residues but in the gamma isoforms
 one PH domain, two SH2 domains, and one SH3 domain are inserted between the
 two PLC-specific domains. The two conserved regions have been shown to be
 important for the catalytic activity. At the C-terminal of the Y-box, there is

a C2 domain (see <PDOC00380>) possibly involved in Ca-dependent membrane attachment.

Profile analysis shows that sequences with significant similarity

to the X-box domain occur also in prokaryotic and trypanosome PI-specific

- 5 phospholipases C. Apart from this region, the prokaryotic enzymes show no similarity to their eukaryotic counterparts.

Two profiles were developed, one covering the X-box, the other the Y-box.

[1] Meldrum E., Parker P.J., Carozzi A.

Biochim. Biophys. Acta 1092:49-71(1991).[2] Rhee S.G., Choi K.D.

- 10 Adv. Second Messenger Phosphoprotein Res. 26:35-61(1992).

[3] Rhee S.G., Choi K.D. J. Biol. Chem. 267:12393-12396(1992).

[4] Sternweis P.C., Smrcka A.V. Trends Biochem. Sci. 17:502-506(1992).

- 15 413. (PI-PLC-Y) Phosphatidylinositol-specific phospholipase C profiles
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- 5 [1] Meldrum E., Parker P.J., Carozzi A.
Biochim. Biophys. Acta 1092:49-71(1991).[2] Rhee S.G., Choi K.D.
Adv. Second Messenger Phosphoprotein Res. 26:35-61(1992).
- [3] Rhee S.G., Choi K.D. J. Biol. Chem. 267:12393-12396(1992).
- [4] Sternweis P.C., Smrcka A.V. Trends Biochem. Sci. 17:502-506(1992).

10

414. (PK) Pyruvate kinase active site signature

Pyruvate kinase (EC 2.7.1.40) (PK) [1] catalyzes the final step in glycolysis, the conversion of phosphoenolpyruvate to pyruvate with the concomitant phosphorylation of ADP to ATP. PK requires both magnesium and potassium ions for its activity. PK is found in all living organisms. In vertebrates there are four, tissues specific, isozymes: L (liver), R (red cells), M1 (muscle, heart, and brain), and M2 (early fetal tissues). In *Escherichia coli* there are two isozymes: PK-I (gene *pykF*) and PK-II (gene *pykA*). All PK isozymes seem to be tetramers of identical subunits of about 500 amino acid residues.

As a signature pattern for PK a conserved region was selected that includes a lysine residue which seems to be the acid/base catalyst responsible for the interconversion of pyruvate and enolpyruvate, and a glutamic acid residue implicated in the binding of the magnesium ion.

25

Consensus pattern: [LIVAC]-x-[LIVM](2)-[SAPCV]-K-[LIV]-E-[NKRST]-x-[DEQHS]-[GSTA]-[LIVM] [K is the active site residue] [E is a magnesium ligand]

- [1] Muirhead H. Biochem. Soc. Trans. 18:193-196(1990).

30

415. (PLDc) Phospholipase D. Active site motif

Phosphatidylcholine-hydrolyzing phospholipase D (PLD) isoforms are activated by ADP-ribosylation factors (ARFs). PLD produces phosphatidic

acid from phosphatidylcholine, which may be essential for the formation of certain types of transport vesicles or may be constitutive vesicular transport to signal transduction pathways.

PC-hydrolyzing PLD is a homologue of cardiolipin synthase,

5 phosphatidylserine synthase, bacterial PLDs, and viral proteins.

Each of these appears to possess a domain duplication which is apparent by the presence of two motifs containing well-conserved histidine, lysine, and/or asparagine residues which may contribute to the active site. aspartic acid. An E. coli endonuclease (nuc) and similar proteins appear

10 to be PLD homologues but possess only one of these motifs.

The profile contained here represents only the putative active site regions, since an accurate multiple alignment of the repeat units has not been achieved.

Number of members: 139

15 [1]

Medline: 96303814

A novel family of phospholipase D homologues that includes phospholipid synthases and putative endonucleases: identification of duplicated repeats and potential active
20 site residues.

Ponting CP, Kerr ID;

Protein Sci 1996;5:914-922.

[2]Medline: 96334293

A duplicated catalytic motif in a new superfamily of

25 phosphohydrolases and phospholipid synthases that includes poxvirus envelope proteins.

Koonin EV;

Trends Biochem Sci 1996;21:242-243.

[3]Medline: 94327597

30 Cloning and expression of phosphatidylcholine-hydrolyzing phospholipase D from Ricinus communis L.

Wang X, Xu L, Zheng L;

J Biol Chem 1994;269:20312-20317.

[4]Medline: 97386825

Regulation of eukaryotic phosphatidylinositol-specific
phospholipase C and phospholipase D.

Singer WD, Brown HA, Sternweis PC;

Annu Rev Biochem 1997;66:475-509.

5

416. (PMI type1) Phosphomannose isomerase type I signatures

Phosphomannose isomerase (EC 5.3.1.8) (PMI) [1,2] is the enzyme that catalyzes the interconversion of mannose-6-phosphate and fructose-6-phosphate. In eukaryotes, it is involved in the synthesis of GDP-mannose which is a constituent of N- and O-linked glycans as well as GPI anchors. In prokaryotes, it is involved in a variety of pathways including capsular polysaccharide biosynthesis and D-mannose metabolism.

10

Three classes of PMI have been defined on the basis of sequence similarities [1]. The first class comprises all known eukaryotic PMI as well as the enzyme encoded by the *manA* gene in enterobacteria such as *Escherichia coli*. Class I PMI's are proteins of about 42 to 50 Kd which bind a zinc ion essential for their activity.

15

As signature patterns for class I PMI, two conserved regions were selected. The first one is located in the N-terminal section of these proteins, the second in the C-terminal half. Both patterns contain a residue involved [3] in the binding of the zinc ion.

20

Consensus pattern: Y-x-D-x-N-H-K-P-E [E is a zinc ligand]

25

-Consensus pattern: H-A-Y-[LIVM]-x-G-x(2)-[LIVM]-E-x-M-A-x-S-D-N-x-[LIVM]-R-A-G-x-T-P-K [H is a zinc ligand]

[1] Proudfoot A.E.I., Turcatti G., Wells T.N.C., Payton M.A., Smith D.J. Eur. J. Biochem. 219:415-423(1994).

30

[2] Coulin F., Magnenat E., Proudfoot A.E.I., Payton M.A., Scully P., Wells T.N.C. Biochemistry 32:14139-14144(1993).

[3] Cleasby A., Wonacott A., Skarzynski T., Hubbard R.E., Davies G.J., Proudfoot A.E.I., Bernard A.R., Payton M.A., Wells T.N.C. Nat. Struct. Biol. 3:470-479(1996).

417. (PNP UDP 1) Purine and other phosphorylases family 1 signature

The following phosphorylases belongs to the same family:

- Purine nucleoside phosphorylase (EC 2.4.2.1) (PNP) from most bacteria (gene deoD). This enzyme catalyzes the cleavage of guanosine or inosine to respective bases and sugar-1-phosphate molecules [1].

- Uridine phosphorylase (EC 2.4.2.3) (UdRPase) from bacteria (gene udp) and mammals. Catalyzes the cleavage of uridine into uracil and ribose-1-phosphate. The products of the reaction are used either as carbon and energy sources or in the rescue of pyrimidine bases for nucleotide synthesis [2].

- 5'-methylthioadenosine phosphorylase (EC 2.4.2.28) (MTA phosphorylase) from *Sulfolobus solfataricus* [3].

As a signature pattern, a conserved region was selected in the central part of these enzymes.

Consensus pattern: [GST]-x-G-[LIVM]-G-x-[PA]-S-x-[GSTA]-I-x(3)-E-L

-Note: it should be noted that mammalian and some bacterial PNP as well as eukaryotic MTA phosphorylase belong to a different family of phosphorylases (see <PDOC00954>).

[1] Takehara M., Ling F., Izawa S., Inoue Y., Kimura A. Biosci. Biotechnol. Biochem. 59:1987-1990(1995).

[2] Watanabe S.-I., Hino A., Wada K., Eliason J.F., Uchida T. J. Biol. Chem. 270:12191-12196(1995).

[3] Cacciapuotì G., Porcelli M., Bertoldo C., De Rosa M., Zappia V. J. Biol. Chem. 269:24762-24769(1994).

418. (PP2C) Protein phosphatase 2C signature

Protein phosphatase 2C (PP2C) is one of the four major classes of mammalian serine/threonine specific protein phosphatases (EC 3.1.3.16). PP2C [1] is a monomeric enzyme of about 42 Kd which shows broad substrate specificity and is dependent on divalent cations (mainly manganese and magnesium) for its activity. Its exact physiological role is still unclear. Three isozymes are

currently known in mammals: PP2C- α , - β and - γ . In yeast, there are at least four PP2C homologs: phosphatase PTC1 [2] which has weak tyrosine phosphatase activity in addition to its activity on serines, phosphatases PTC2 and PTC3, and hypothetical protein YBR125c. Isozymes of PP2C are also known from *Arabidopsis thaliana* (ABI1, PPH1), *Caenorhabditis elegans* (FEM-2, F42G9.1, T23F11.1), *Leishmania chagasi* and *Paramecium tetraurelia*. In *Arabidopsis thaliana*, the kinase associated protein phosphatase (KAPP) [3] is an enzyme that dephosphorylates the Ser/Thr receptor-like kinase RLK5 and which contains a C-terminal PP2C domain.

PP2C does not seem to be evolutionary related to the main family of serine/threonine phosphatases: PP1, PP2A and PP2B. However, it is significantly similar to the catalytic subunit of pyruvate dehydrogenase phosphatase (EC 3.1.3.43) (PDPC) [4], which catalyzes dephosphorylation and concomitant reactivation of the α subunit of the E1 component of the pyruvate dehydrogenase complex. PDPC is a mitochondrial enzyme and, like PP2C, is magnesium-dependent.

As a signature pattern, the best conserved region was selected which is located in the N-terminal part and contains a perfectly conserved tripeptide. This region includes a conserved aspartate residue involved in divalent cation binding [5].

Consensus pattern: [LIVMFY]-[LIVMFYA]-[GSAC]-[LIVM]-[FYC]-D-G-H-[GAV]

-Note: PP2C belongs [6] to a superfamily which also includes bacterial proteins such as *Bacillus spoIIE*, *rsbU* and *rsbW*, *Synechocystis* PCC 6803 *icfG* as well as a domain in fungal adenylate cyclases.

[1] Wenk J., Trompeter H.-I., Pettrich K.-G., Cohen P.T.W., Campbell D.G., Mieskes G. FEBS Lett. 297:135-138(1992).

[2] Maeda T., Tsai A.Y.M., Saito H. Mol. Cell. Biol. 13:5408-5417(1993).

[3] Stone J.M., Collinge M.A., Smith R.D., Horn M.A., Walker J.C. Science 266:793-795(1994).

[4] Lawson J.E., Niu X.-D., Browning K.S., Trong H.L., Yan J., Reed L.J. Biochemistry 32:8987-8993(1993).

[5] Das A.K., Helps N.R., Cohen P.T.W., Barford D. EMBO J. 24:6798-6809(1996).

[6] Bork P., Brown N.P., Hegyi H., Schultz J. Protein Sci. 5:1421-1425(1996).

419. (PPTA) Protein prenyltransferases alpha subunit repeat signature

5 Protein prenyltransferases catalyze the transfer of an isoprenyl moiety to a cysteine four residues from the C-terminus of several proteins. They are heterodimeric enzymes consisting of alpha and beta subunits. The alpha subunit is thought to participate in a stable complex with the isoprenyl substrate; the beta subunit binds the peptide substrate. Distinct protein
10 prenyltransferases might share a common alpha subunit. Both the alpha and beta subunit show repetitive sequence motifs [1]. These repeats have distinct structural and functional implications and are unrelated to each other. Known protein prenyltransferase alpha subunits are:

- Mammalian protein farnesyltransferase alpha subunit.
 - 15 - Yeast protein RAM2, a protein farnesyltransferase alpha subunit.
 - Yeast protein BET4, a protein geranylgeranyltransferase alpha subunit.
- The conserved domain of the alpha subunit consists of about 34 amino acids and is repeated five times. It contains an invariant tryptophan possibly involved in heterodimerization with the conserved phenylalanines in the repeated
20 domains of the beta subunits, via hydrophobic bonds. The signature pattern for this domain is centered on the invariant tryptophan.

Consensus pattern: [PSIAV]-x-[NDFV]-[NEQIY]-x-[LIVMAGP]-W-[NQSTHF]-[FYHQ]-[LIVMR]

[1] Boguski M.S., Murray A.W., Powers S. New Biol. 4:408-411(1992).

420. (PR55) Protein phosphatase 2A regulatory subunit PR55 signatures

30 Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase involved in many aspects of cellular function including the regulation of metabolic enzymes and proteins involved in signal transduction. PP2A is a trimeric enzyme that consists of a core composed of a catalytic subunit associated with a 65 Kd regulatory subunit (PR65), also called subunit A; this complex then

associates with a third variable subunit (subunit B), which confers distinct properties to the holoenzyme [1]. One of the forms of the variable subunit is a 55 Kd protein (PR55) which is highly conserved in mammals - where three isoforms are known to exist -, *Drosophila* and yeast (gene CDC55). This subunit could perform a substrate recognition function or be responsible for targeting the enzyme complex to the appropriate subcellular compartment. As signature patterns, two perfectly conserved sequences of 15 residues were selected; one located in the N-terminal region, the other in the center of the protein.

Consensus pattern: E-F-D-Y-L-K-S-L-E-I-E-E-K-I-N

Consensus pattern: N-[AG]-H-[TA]-Y-H-I-N-S-I-S-[LIVM]-N-S-D

[1] Mayer-Jaekel R., Hemmings B.A. Trends Cell Biol. 4:287-291(1994).

421. N-(5'phosphoribosyl)anthranilate (PRA) isomerase

[1] Wilmanns M, Priestle JP, Niermann T, Jansonius JN;
J Mol Biol 1992;223:477-507.

422. (PRK) Phosphoribulokinase signature

Phosphoribulokinase (EC 2.7.1.19) (PRK) [1,2] is one of the enzymes specific to the Calvin's reductive pentose phosphate cycle which is the major route by which carbon dioxide is assimilated and reduced by autotrophic organisms. PRK catalyzes the ATP-dependent phosphorylation of ribulose 5-phosphate into ribulose 1,5-bisphosphate which is the substrate for RubisCO.

PRK's of diverse origins show different properties with respect to the size of the protein, the subunit structure, or the enzymatic regulation. However an alignment of the sequences of PRK from plants, algae, photosynthetic and chemoautotrophic bacteria shows that there are a few regions of sequence similarity. As a signature pattern one of these regions was selected.

Consensus pattern: K-[LIVM]-x-R-D-x(3)-R-G-x-[ST]-x-E

- [1] Kossmann J., Klintworth R., Bowien B. Gene 85:247-252(1989).
[2] Gibson J.L., Chen J.-H., Tower P.A., Tabita F.R. Biochemistry 29:8085-8093(1990).

5

423. (PRPP synt) Phosphoribosyl pyrophosphate synthetase signature
Phosphoribosyl pyrophosphate synthetase (EC 2.7.6.1) (PRPP synthetase)
catalyzes the formation of PRPP from ATP and ribose 5-phosphate. PRPP is then
used in various biosynthetic pathways, as for example in the formation of
purines, pyrimidines, histidine and tryptophan. PRPP synthetase requires
inorganic phosphate and magnesium ions for its stability and activity.

10

In mammals, three isozymes of PRPP synthetase are found; in yeast there are at
least four isozymes.

15

As a signature pattern for this enzyme, a very conserved region was selected
that has been suggested to be involved in binding divalent cations [1]. This
region contains two conserved aspartic acid residues as well as a histidine,
which are all potential ligands for a cation such as magnesium.

20

Consensus pattern: D-[LI]-H-[SA]-x-Q-[IMST]-[QM]-G-[FY]-F-x(2)-P-[LIVMFC]-D

- [1] Bower S.G., Harlow K.W., Switzer R.L., Hoven-Jensen B. J. Biol. Chem. 264:10287-
10291(1989).

25

424. (PRTP) Herpesvirus processing and transport protein

The members of this family are associate with capsid intermediates during packaging of the
virus.

Number of members: 31

[1]

30

Medline: 98362148

Herpes simplex virus type 1 cleavage and packaging proteins
UL15 and UL28 are associated with B but not C capsids during
packaging. Yu D, Weller SK;
J Virol 1998;72:7428-7439.

425. Photosystem I psaG / psaK (PSI PSAK) proteins signature

Photosystem I (PSI) [1] is an integral membrane protein complex that uses light energy to mediate electron transfer from plastocyanin to ferredoxin. It is found in the chloroplasts of plants and cyanobacteria. PSI is composed of at least 14 different subunits, two of which PSI-G (gene psaG) and PSI-K (gene psaK) are small hydrophobic proteins of about 7 to 9 Kd and evolutionary related [2]. Both seem to contain two transmembrane regions. Cyanobacteria seem to encode only for PSI-K.

As a signature pattern, the best-conserved region was selected which seems to correspond to the second transmembrane region.

-Consensus pattern: [GT]-F-x-[LIVM]-x-[DEA]-x(2)-[GA]-x-[GTA]-[SA]-x-G-H-x-[LIVM]-[GA]

[1] Golbeck J.H. Biochim. Biophys. Acta 895:167-204(1987).

[2] Kjaerulff S., Andersen B., Nielsen V.S., Moller B.L., Okkels J.S. J. Biol. Chem. 268:18912-18916(1993).

426. PTR2 family proton/oligopeptide symporters signatures

A family of eukaryotic and prokaryotic proteins that seem to be mainly involved in the intake of small peptides with the concomitant uptake of a proton has been recently characterized [1,2]. Proteins that belong to this family are:

- Fungal peptide transporter PTR2.
- Mammalian intestine proton-dependent oligopeptide transporter PeptT1.
- Mammalian kidney proton-dependent oligopeptide transporter PeptT2.
- Drosophila opt1.
- Arabidopsis thaliana peptide transporters PTR2-A and PTR2-B (also known as the histidine transporting protein NTR1).
- Arabidopsis thaliana proton-dependent nitrate/chlorate transporter CHL1.
- Lactococcus proton-dependent di- and tri-peptide transporter dtpT.
- Caenorhabditis elegans hypothetical protein C06G8.2.
- Caenorhabditis elegans hypothetical protein F56F4.5.

- *Caenorhabditis elegans* hypothetical protein K04E7.2.

- *Escherichia coli* hypothetical protein ybGH.

- *Escherichia coli* hypothetical protein ydGR.

- *Escherichia coli* hypothetical protein yhiP.

5 - *Escherichia coli* hypothetical protein yjdL.

- *Bacillus subtilis* hypothetical protein yclF.

These integral membrane proteins are predicted to comprise twelve transmembrane regions. As signature patterns, two of the best conserved regions were selected. The first is a region that includes the end of the second transmembrane region, a cytoplasmic loop as well as the third transmembrane region. The second pattern corresponds to the core of the fifth transmembrane region.

-Consensus pattern: [GA]-[GAS]-[LIVMFYWA]-[LIVM]-[GAS]-D-x-[LIVMFYWT]-

15 [LIVMFYW]-G-x(3)-[TAV]-[IV]-x(3)-[GSTAV]-x-[LIVMF]-x(3)-[GA]

-Consensus pattern: [FYT]-x(2)-[LMFY]-[FYV]-[LIVMFYWA]-x-[IVG]-N-[LIVMAG]-G-[GSA]-[LIMF]

[1] Paulsen I.T., Skurray R.A. Trends Biochem. Sci. 19:404-404(1994).

20 [2] Steiner H.-Y., Naider F., Becker J.M. Mol. Microbiol. 16:825-834(1995).

427. Pumilio-family RNA binding domains (aka PUM-HD, Pumilio homology domain)

25 Puf domains are necessary and sufficient for sequence specific

RNA binding in fly Pumilio and worm FBF-1 and FBF-2. Both proteins

function as translational repressors in early embryonic development

by binding sequences in the 3' UTR of target mRNAs (e.g. the

nanos response element (NRE) in fly Hunchback mRNA, or the point

30 mutation element (PME) in worm fem-3 mRNA). Other proteins that contain Puf domains are

also plausible RNA binding proteins. JSN1_YEAST, for instance, appears to also contain a

single RRM domain by HMM analysis.

Puf domains usually occur as a tandem repeat of 8 domains.

The Pfam model does not necessarily recognize all 8 domains in

all sequences; some sequences appear to have 5 or 6 domains on initial analysis, but further analysis suggests the presence of additional divergent domains.

- 5 [1] Zhang B, Gallegos M, Puoti A, Durkin E, Fields S, Kimble J, Wickens MP. *Nature* 1997;390:477-484. [2] Zamore PD, Williamson JR, Lehmann R. *RNA* 1997;3:1421-1433.

- 10 428. PWWP domain. The PWWP domain is named after a conserved Pro-Trp-Trp-Pro motif. The function of the domain is currently unknown. Number of members: 19

- [1] Medline: 98282232. WHSC1, a 90 kb SET domain-containing gene, expressed in early development and homologous to a *Drosophila* dysmorphia gene maps in the Wolf-Hirschhorn syndrome critical region and is fused to IgH in t(4;14) multiple myeloma. Stec I, Wright TJ, van Ommen GJB, de Boer PAJ, van Haeringen A, Moorman AFM, Altherr MR, den Dunnen JT; *Hum Mol Genet* 1998;7:1071-1082.

- 20 429. PX domain
Eukaryotic domain of unknown function present in phox proteins, PLD isoforms, a PI3K isoform.

Number of members: 71

[1]

- 25 Medline: 97084820
Novel domains in NADPH oxidase subunits, sorting nexins, and PtdIns 3-kinases: binding partners of SH3 domains?
Ponting CP;
Protein Sci 1996;5:2353-2357.

30

430. ParA family ATPase

[1]

Medline: 91141297

A family of ATPases involved in active partitioning of diverse bacterial plasmids.

Motallebi-Veshareh M, Rouch DA, Thomas CM;

Mol Microbiol 1990;4:1455-1463.

5 Number of members: 122

431. (Parvo coat) Parvovirus coat protein. 72 members.

10

432. Pectinesterase signatures

Pectinesterase (EC 3.1.1.11) (pectin methylesterase) catalyzes the hydrolysis of pectin into pectate and methanol. In plants, it plays an important role in cell wall metabolism during fruit ripening. In plant bacterial pathogens such as *Erwinia carotovora* and in fungal pathogens such as *Aspergillus niger*, pectinesterase is involved in maceration and soft-rotting of plant tissue.

Prokaryotic and eukaryotic pectinesterases share a few regions of sequence similarity [1,2,3]. two of these regions were selected as signature patterns.

The first is based on a region in the N-terminal section of these enzymes; it contains a conserved tyrosine which may play a role in the catalytic mechanism [3]. The second pattern corresponds to the best conserved region, an octapeptide located in the central part of these enzymes.

-Consensus pattern: [GSTNP]-x(6)-[FYVHR]-[IVN]-[KEP]-x-G-[STIVKRO]-Y-

[DNQKRMV]-[EP]-x(3)-[LIMVA]

-Consensus pattern: [IV]-x-G-[STAD]-[LIVT]-D-[FYI]-[IV]-[FSN]-G

[1] Ray J., Knapp J., Grierson D., Bird C., Schuch W. Eur. J. Biochem. 174:119-124(1988).

[2] Plastow G.S. Mol. Microbiol. 2:247-254(1988).

[3] Markovic O., Joernvall H. Protein Sci. 1:1288-1292(1992).

433. Pentapeptide repeats (8 copies)

These repeats are found in many cyanobacterial proteins.

The repeats were first identified in hglK [1]. The function of these repeats is unknown.

The structure of this repeat has been predicted to be a beta-helix [2].

- 5 The repeat can be approximately described as A(D/N)LXX, where X can be any amino acid. Number of members: 75

[1]

Medline: 96062225

- 10 The hglK gene is required for localization of heterocyst-specific glycolipids in the cyanobacterium *Anabaena* sp. strain PCC 7120.

Black K, Buikema WJ, Haselkorn R;
J Bacteriol 1995;177:6440-6448.

[2]Medline: 98318059

- 15 Structure and distribution of pentapeptide repeats in bacteria.

Bateman A, Murzin A, Teichmann SA;
Protein Sci 1998;7:1477-1480.

[3]Medline: 98316713

- 20 Characterisation of an Arabidopsis cDNA encoding a thylakoid lumen protein related to a novel 'pentapeptide repeat' family of proteins.

Kieselbach T, Mant A, Robinson C, Schroder WP;
FEBS Lett 1998;428:241-244.

25

434. Polypeptide deformylase

[1]

Medline: 97002011

- 30 A new subclass of the zinc metalloproteases superfamily revealed by the solution structure of peptide deformylase.

Meinzel T, Blanquet S, Dardel F;
J Mol Biol 1996;262:375-386.

[2]Medline: 98332750

Solution structure of nickel-peptide deformylase.

Dardel F, Ragusa S, Lazennec C, Blanquet S, Meinnel T;

J Mol Biol 1998;280:501-513.

Number of members: 21

5

435. Peptidyl-tRNA hydrolase signatures

Peptidyl-tRNA hydrolase (EC 3.1.1.29) (PTH) is a bacterial enzyme that cleaves peptidyl-tRNA or N-acyl-aminoacyl-tRNA to yield free peptides or N-acyl-amino acids and tRNA. The natural substrate for this enzyme may be peptidyl-tRNA which drop off the ribosome during protein synthesis [1,2]. Bacterial PTH has been found [2,3] to be evolutionary related to yeast hypothetical protein YHR189w.

10

PTH and YHR189w are proteins of about 200 amino acid residues. As signature patterns, two conserved regions were selected that each contain an histidine.

15

The first of these regions is located in the N-terminal section, the other in the central part.

-Consensus pattern: [FY]-x(2)-T-R-H-N-x-G-x(2)-[LIVMFA](2)-[DE]

20

-Consensus pattern: [GS]-x(3)-H-N-G-[LIVM]-[KR]-[DNS]-[LIVMT]

[1] Garcia-Villegas M.R., De La Vega F.M., Galindo J.M., Segura M., Buckingham R.H., Guarneros G. EMBO J. 10:3549-3555(1991).

[2] De La Vega F.M., Galindo J.M., Old I.G., Guarneros G. Gene 169:97-100(1996).

25

[3] Ouzounis C., Bork P., Casari G., Sander C. Protein Sci. 4:2424-2428(1995).

436. (Peptidase M17) Cytosol aminopeptidase signature

Cytosol aminopeptidase is a eukaryotic cytosolic zinc-dependent exopeptidase that catalyzes the removal of unsubstituted amino-acid residues from the N-terminus of proteins. This enzyme is often known as leucine aminopeptidase (EC 3.4.11.1) (LAP) but has been shown [1] to be identical with prolyl aminopeptidase (EC 3.4.11.5). Cytosol aminopeptidase is a hexamer of identical chains, each of which binds two zinc ions.

30

Cytosol aminopeptidase is highly similar to *Escherichia coli* pepA, a manganese dependent aminopeptidase. Residues involved in zinc ion-binding [2] in the mammalian enzyme are absolutely conserved in pepA where they presumably bind manganese.

- 5 A cytosol aminopeptidase from *Rickettsia prowazekii* [3] and one from *Arabidopsis thaliana* also belong to this family.
- As a signature pattern for these enzymes, a perfectly conserved octapeptide was selected which contains two residues involved in binding metal ions: an aspartate and a glutamate.

10

-Consensus pattern: N-T-D-A-E-G-R-L [The D and the E are zinc/manganese ligands]

-Note: these proteins belong to family M17 in the classification of peptidases [4,E1].

15

[1] Matsushima M., Takahashi T., Ichinose M., Miki K., Kurokawa K., Takahashi K.

Biochem. Biophys. Res. Commun. 178:1459-1464(1991).

[2] Burley S.K., David P.R., Sweet R.M., Taylor A., Lipscomb W.N. J. Mol. Biol. 224:113-140(1992).

[3] Wood D.O., Solomon M.J., Speed R.R. J. Bacteriol. 175:159-165(1993).

[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

20

437. Assemblin (Peptidase family S21)

[1]

Medline: 96399137

- 25 Three-dimensional structure of human cytomegalovirus protease.

Shieh HS, Kurumbail RG, Stevens AM, Stegeman RA, Sturman EJ,

Pak JY, Wittwer AJ, Palmier MO, Wiegand RC, Holwerda BC,

Stallings WC;

- 30 Nature 1996;383:279-282.

Number of members: 29

438. Pollen proteins Ole e I family signature

The following plant pollen proteins, whose biological function is not yet known, are structurally related [1]:

- Olive tree pollen major allergen (Ole e I).

- Tomato anther-specific protein LAT52. - Maize pollen-specific protein ZmC13.

- 5 These proteins are most probably secreted and consist of about 145 residues.

As shown in the following schematic representation, there are six cysteines which are conserved in the sequence of these proteins. They seem to be involved in disulfide bonds.

xxxxxxCxXX

- 10 *****'C': conserved cysteine involved in a disulfide bond.

'*': position of the pattern.

-Consensus pattern: [EQ]-G-x-V-Y-C-D-T-C-R [The two C's are probably involved in disulfide bonds]

15

[1] Villalba M., Batanero E., Lopez-Otin C., Sanchez L.M., Monsalve R.I., Gonzalez De La Pena M.A., Lahoz C., Rodriguez R. Eur. J. Biochem. 216:863-869(1993).

20

439. Pollen allergen

This family contains allergens lol PI, PII and PIII from *Lolium perenne*.

Number of members: 49

[1]

Medline: 90105394

25

Complete primary structure of a *Lolium perenne* (perennial rye grass) pollen allergen, Lol p III: comparison with known Lol p I and II sequences.

Ansari AA, Shenbagamurthi P, Marsh DG;

Biochemistry 1989;28:8665-8670.

30

440. Porphobilinogen deaminase cofactor-binding site

Porphobilinogen deaminase (EC 4.3.1.8), or hydroxymethylbilane synthase, is an enzyme involved in the biosynthesis of porphyrins and related macrocycles. It

catalyzes the assembly of four porphobilinogen (PBG) units in a head to tail fashion to form hydroxymethylbilane.

The enzyme covalently binds a dipyrromethane cofactor to which the PBG subunits are added in a stepwise fashion. In the *Escherichia coli* enzyme (gene hemC), this cofactor has been shown [1] to be bound by the sulfur atom of a cysteine. The region around this cysteine is conserved in porphobilinogen deaminases from various prokaryotic and eukaryotic sources.

-Consensus pattern: E-R-x-[LIVMF]-x(3)-[LIVMF]-x-G-[GSA]-C-x-[IVT]-P-[LIVMF]-

10 [GSA] [C is the cofactor attachment site]

[1] Miller A.D., Hart G.J., Packman L.C., Battersby A.R. Biochem. J. 254:915-918(1988).

15 441. Presenilin

Mutations in presenilin-1 are a major cause of early onset Alzheimer's disease [2]. It has been found that presenilin-1 (Swiss:P49768) binds to beta-catenin in vivo [4]. This family also contains SPE proteins from *C.elegans*.

Number of members: 23

20 [1]

Medline: 98045995

Presenilins and Alzheimer's disease.

Kim TW, Tanzi RE;

Curr Opin Neurobiol 1997;7:683-688.

25 [2]Medline: 98045995

Presenilins and Alzheimer's disease.

Kim TW, Tanzi RE;

Curr Opin Neurobiol 1997;7:683-688.

[3]Medline: 98099802

30 Interaction of presenilins with the filamin family of actin-binding proteins.

Zhang W, Han SW, McKeel DW, Goate A, Wu JY;

J Neurosci 1998;18:914-922.

[4]Medline: 99004850

Destabilisation of beta-catenin by mutations in presenilin-1
potentiates neuronal apoptosis.

Zhang Z, Hartmann H, Do VM, Abramowski D, Sturchler-Pierrat
C, Staufenbiel M, Sommer B, van de Wetering M, Clevers H,
Saftig P, De Strooper B, He X, Yankner BA;
Nature 1998;395:698-702.

442. (Pribosyltran) Purine/pyrimidine phosphoribosyl transferases signature

10 Phosphoribosyltransferases (PRT) are enzymes that catalyze the synthesis of
beta-n-5'-monophosphates from phosphoribosylpyrophosphate (PRPP) and an enzyme
specific amine. A number of PRT's are involved in the biosynthesis of purine,
pyrimidine, and pyridine nucleotides, or in the salvage of purines and
pyrimidines. These enzymes are:

- 15 - Adenine phosphoribosyltransferase (EC 2.4.2.7) (APRT), which is involved in
purine salvage.
- Hypoxanthine-guanine or hypoxanthine phosphoribosyltransferase (EC 2.4.2.8)
(HGPRT or HPRT), which are involved in purine salvage.
- Orotate phosphoribosyltransferase (EC 2.4.2.10) (OPRT), which is involved
20 in pyrimidine biosynthesis.
- Amido phosphoribosyltransferase (EC 2.4.2.14), which is involved in purine
biosynthesis.
- Xanthine-guanine phosphoribosyltransferase (EC 2.4.2.22) (XGPRT), which is
involved in purine salvage.

25 In the sequence of all these enzymes there is a small conserved region which
may be involved in the enzymatic activity and/or be part of the PRPP binding
site [1].

-Consensus pattern: [LIVMFYWCTA]-[LIVM]-[LIVMA]-[LIVMFC]-[DE]-D-[LIVMS]-
30 [LIVM]-[STAVD]-[STAR]-[GAC]-x-[STAR]
-Note: in position 11 of the pattern most of these enzymes have Gly.

[1] Hershey H.V., Taylor M.W. Gene 43:287-293(1986).

443. (Pro CA)

Prokaryotic-type carbonic anhydrases signatures

- 5 Carbonic anhydrases (EC 4.2.1.1) (CA) are zinc metalloenzymes which catalyze the reversible hydration of carbon dioxide. In *Escherichia coli*, CA (gene *cynT*) is involved in recycling carbon dioxide formed in the bicarbonate-dependent decomposition of cyanate by cyanase (gene *cynS*). By this action, it prevents the depletion of cellular bicarbonate [1]. In photosynthetic bacteria and plant chloroplast, CA is essential to inorganic carbon fixation [2].
- 10 Prokaryotic and plant chloroplast CA are structurally and evolutionary related and form a family distinct from the one which groups the many different forms of eukaryotic CA's (see <PDOC00146>). Hypothetical proteins *yadF* from *Escherichia coli* and H11301 from *Haemophilus influenzae* also belong to this family. Two signature patterns were developed for this family of enzymes. Both patterns contain conserved residues that could be involved
- 15 in binding zinc (cysteine and histidine).

-Consensus pattern: C-[SA]-D-S-R-[LIVM]-x-[AP]

-Consensus pattern: [EQ]-Y-A-[LIVM]-x(2)-[LIVM]-x(4)-[LIVMF](3)-x-G-H-x(2)-C-G

- 20 [1] Guilloton M.B., Korte J.J., Lamblin A.F., Fuchs J.A., Anderson P.M. J. Biol. Chem. 267:3731-3734(1992).
- [2] Fukuzawa H., Suzuki E., Komukai Y., Miyachi S. Proc. Natl. Acad. Sci. U.S.A. 89:4437-4441(1992).

25

444. (Prolyl_oligopep)

Prolyl oligopeptidase family serine active site

30

The prolyl oligopeptidase family [1,2,3] consist of a number of evolutionary related peptidases whose catalytic activity seems to be provided by a charge relay system similar to that of the trypsin family of serine proteases, but which evolved by independent convergent evolution. The known members of this family are listed below.

- Prolyl endopeptidase (EC 3.4.21.26) (PE) (also called post-proline cleaving enzyme). PE is an enzyme that cleaves peptide bonds on the C-terminal side of prolyl residues. The sequence of PE has been obtained from a mammalian species (pig) and from bacteria (*Flavobacterium meningosepticum* and *Aeromonas hydrophila*); there is a high degree of sequence

conservation between these sequences.

- *Escherichia coli* protease II (EC 3.4.21.83) (oligopeptidase B) (gene *prtB*) which cleaves peptide bonds on the C-terminal side of lysyl and arginyl residues.

- Dipeptidyl peptidase IV (EC 3.4.14.5) (DPP IV). DPP IV is an enzyme that removes N-terminal dipeptides sequentially from polypeptides having unsubstituted N-termini provided that the penultimate residue is proline.

- Yeast vacuolar dipeptidyl aminopeptidase A (DPAP A) (gene: *STE13*) which is responsible for the proteolytic maturation of the alpha-factor precursor.

- Yeast vacuolar dipeptidyl aminopeptidase B (DPAP B) (gene: *DAP2*).

- Acylamino-acid-releasing enzyme (EC 3.4.19.1) (acyl-peptide hydrolase).

This enzyme catalyzes the hydrolysis of the amino-terminal peptide bond of an N-acetylated protein to generate a N-acetylated amino acid and a protein with a free amino-terminus.

A conserved serine residue has experimentally been shown (in *E.coli* protease II as well as in pig and bacterial PE) to be necessary for the catalytic mechanism. This serine, which is part of the catalytic triad (Ser, His, Asp), is generally located about 150 residues away from the C-terminal extremity of these enzymes (which are all proteins that contains about 700 to 800 amino acids).

Consensus pattern: D-x(3)-A-x(3)-[LIVMFYW]-x(14)-G-x-S-x-G-G-[LIVMFYW](2) [S is the active site residue] Sequences known to belong to this class detected by the pattern ALL, except for yeast DPAP A.

Note: these proteins belong to families S9A/S9B/S9C in the classification of peptidases [4].

[1] Rawlings N.D., Polgar L., Barrett A.J. *Biochem. J.* 279:907-911(1991).

[2] Barrett A.J., Rawlings N.D.

[3] Polgar L., Szabo E.

[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

5

445. (Pterin 4a)

Pterin 4 alpha carbinolamine dehydratase

10

Pterin 4 alpha carbinolamine dehydratase is aka DCoH (dimerisation cofactor of hepatocyte nuclear factor 1-alpha).

Number of members: 11

15

[1] Cronk JD, Endrizzi JA, Alber T; Medline: 97052967 High-resolution structures of the bifunctional enzyme and transcriptional coactivator DCoH and its complex with a product analogue." Protein Sci 1996;5:1963-1972.

446. (Pyridox oxidase)

20

Pyridoxamine 5'-phosphate oxidase signature

25

Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5) is a FMN flavoprotein involved in the de novo synthesis of pyridoxine (vitamin B6) and pyridoxal phosphate. It oxidizes pyridoxamine-5-P (PMP) and pyridoxine-5-P (PNP) to pyridoxal-5-P. The sequences of the enzyme from bacterial (genes *pdxH* or *fprA*) [1] and fungal (gene *PDX3*) [2] sources show that this protein has been highly conserved throughout evolution. PdxH is evolutionary related [3] to one of the enzymes in the phenazine biosynthesis protein pathway, *phzD* (also known as *phzG*). As a signature pattern, a highly conserved region was selected located in the C-terminal part of these enzymes.

30

-Consensus pattern: [LIVF]-E-F-W-[QHG]-x(4)-R-[LIVM]-H-[DNE]-R

[1] Lam H.-M., Winkler M.E. J. Bacteriol. 174:6033-6045(1992).

[2] Loubbardi A., Karst F., Guilloton M., Marcireau C. J. Bacteriol. 177:1817-1823(1995).

[3] Pierson L.S. III, Gaffney T., Lam S., Gong F. FEMS Microbiol. Lett. 134:299-307(1995).

5 447. (Pyrophosphatase)

Inorganic pyrophosphatase signature

Inorganic pyrophosphatase (EC 3.6.1.1) (PPase) [1,2] is the enzyme responsible for the hydrolysis of pyrophosphate (PPi) which is formed principally as the product of the many biosynthetic reactions that utilize ATP. All known PPases require the presence of divalent metal cations, with magnesium conferring the highest activity. Among other residues, a lysine has been postulated to be part or close to the active site. PPases have been sequenced from bacteria such as *Escherichia coli* (homohexamer), thermophilic bacteria PS-3 and *Thermus thermophilus*, from the archaeobacteria *Thermoplasma acidophilum*, from fungi (homodimer), from a plant, and from bovine retina. In yeast, a mitochondrial isoform of PPase has been characterized which seems to be involved in energy production and whose activity is stimulated by uncouplers of ATP synthesis.

The sequences of PPases share some regions of similarities. As signature patterns a region was selected that contains three conserved aspartates that are involved in the binding of cations.

-Consensus pattern: D-[SGDN]-D-[PE]-[LIVMF]-D-[LIVMGAC]

[The three D's bind divalent metal cations]

[1] Lahti R., Kolakowski L.F. Jr., Heinonen J., Vihinen M., Pohjanoksa K., Cooperman B.S. Biochim. Biophys. Acta 1038:338-345(1990).

[2] Cooperman B.S., Baykov A.A., Lahti R. Trends Biochem. Sci. 17:262-266(1992).

448. (Peptidase S26)

Signal peptidases I signatures.

Signal peptidases (SPases) [1] (aka leader peptidases) remove the signal peptides from secretory proteins. In prokaryotes three types of SPases are known: type I (gene *lepB*) which is responsible for the processing of the majority of exported pre-proteins; type II (gene *lsp*) which only process lipoproteins, and a third type involved in the processing of pili subunits.

5 SPase I (EC 3.4.21.89) is an integral membrane protein that is anchored in the cytoplasmic membrane by one (in *B. subtilis*) or two (in *E. coli*) N-terminal transmembrane domains with the main part of the protein protruding in the periplasmic space. Two residues have been shown [2,3] to be essential for the catalytic activity of SPase I: a serine and a lysine. SPase I is evolutionary related to the yeast mitochondrial inner membrane protease subunit 1 and 2
10 (genes *IMP1* and *IMP2*) which catalyze the removal of signal peptides required for the targeting of proteins from the mitochondrial matrix, across the inner membrane, into the inter-membrane space [4]. In eukaryotes the removal of signal peptides is effected by an oligomeric enzymatic complex composed of at least five subunits: the signal peptidase complex (SPC). The SPC is located in the endoplasmic reticulum membrane. Two
15 components of mammalian SPC, the 18 Kd (SPC18) and the 21 Kd (SPC21) subunits as well as the yeast SEC11 subunit have been shown [5] to share regions of sequence similarity with prokaryotic SPases I and yeast *IMP1/IMP2*. Three signature patterns have been developed for these proteins. The first signature contains the putative active site serine, the second signature contains the putative active site lysine which is not conserved in the SPC subunits, and the
20 third signature corresponds to a conserved region of unknown biological significance which is located in the C-terminal section of all these proteins.

Consensus pattern: [GS]-x-S-M-x-[PS]-[AT]-[LF] [S is an active site residue]-

Consensus pattern: K-R-[LIVMSTA](2)-G-x-[PG]-G-[DE]-x-[LIVM]-x-[LIVMFY] [K is an
25 active site residue]-

Consensus pattern: [LIVMFYW](2)-x(2)-G-D-[NH]-x(3)-[SND]-x(2)-[SG]-

[1] Dalbey R.E., von Heijne G. Trends Biochem. Sci. 17:474-478(1992).[2] Sung M., Dalbey R.E. J. Biol. Chem. 267:13154-13159(1992).[3] Black M.T. J. Bacteriol. 175:4957-
30 4961(1993).[4] Nunnari J., Fox T.D., Walter P. Science 262:1997-2004(1993).[5] van Dijk J.M., de Jong A., Vehmaanperä J., Venema G., Bron S. EMBO J. 11:2819-2828(1992).[6] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).[E1]

449. (Peptidase C1) Eukaryotic thiol (cysteine) proteases active sites. Eukaryotic thiol proteases (EC 3.4.22.-) [1] are a family of proteolytic enzymes which contain an active site cysteine. Catalysis proceeds through a thioester intermediate and is facilitated by a nearby histidine side chain; an asparagine completes the essential catalytic triad. The proteases

5 which are currently known to belong to this family are listed below (references are only provided for recently determined sequences). - Vertebrate lysosomal cathepsins B (EC 3.4.22.1), H (EC 3.4.22.16), L (EC 3.4.22.15), and S (EC 3.4.22.27) [2]. - Vertebrate lysosomal dipeptidyl peptidase I (EC 3.4.14.1) (also known as cathepsin C) [2]. - Vertebrate calpains (EC 3.4.22.17). Calpains are intracellular calcium-activated thiol protease that

10 contain both a N-terminal catalytic domain and a C-terminal calcium-binding domain. - Mammalian cathepsin K, which seems involved in osteoclastic bone resorption [3]. - Human cathepsin O [4]. - Bleomycin hydrolase. An enzyme that catalyzes the inactivation of the antitumor drug BLM (a glycopeptide). - Plant enzymes: barley aleurain (EC 3.4.22.16), EP-B1/B4; kidney bean EP-C1, rice bean SH-EP; kiwi fruit actinidin (EC 3.4.22.14); papaya latex papain (EC 3.4.22.2), chymopapain (EC 3.4.22.6), caricain (EC 3.4.22.30), and

15 proteinase IV (EC 3.4.22.25); pea turgor-responsive protein 15A; pineapple stem bromelain (EC 3.4.22.32); rape COT44; rice oryzain alpha, beta, and gamma; tomato low-temperature induced, Arabidopsis thaliana A494, RD19A and RD21A. - House-dust mites allergens DerP1 and EurM1. - Cathepsin B-like proteinases from the worms *Caenorhabditis elegans* (genes *gcp-1*, *cpr-3*, *cpr-4*, *cpr-5* and *cpr-6*), *Schistosoma mansoni* (antigen SM31) and

20 *Japonica* (antigen SJ31), *Haemonchus contortus* (genes AC-1 and AC-2), and *Ostertagia ostertagi* (CP-1 and CP-3). - Slime mold cysteine proteinases CP1 and CP2. - Cruzipain from *Trypanosoma cruzi* and *brucei*. - Trophozoite cysteine proteinase (TCP) from various *Plasmodium* species. - Proteases from *Leishmania mexicana*, *Theileria annulata* and *Theileria*

25 *parva*. - Baculoviruses cathepsin-like enzyme (v-cath). - *Drosophila* small optic lobes protein (gene *sol*), a neuronal protein that contains a calpain-like domain. - Yeast thiol protease BLH1/YCP1/LAP3. - *Caenorhabditis elegans* hypothetical protein C06G4.2, a calpain-like protein. Two bacterial peptidases are also part of this family: - Aminopeptidase C from *Lactococcus lactis* (gene *pepC*) [5]. - Thiol protease *tpf* from *Porphyromonas gingivalis*.

30 Three other proteins are structurally related to this family, but may have lost their proteolytic activity. - Soybean oil body protein P34. This protein has its active site cysteine replaced by a glycine. - Rat testin, a sertoli cell secretory protein highly similar to cathepsin L but with the active site cysteine is replaced by a serine. Rat testin should not be confused with mouse testin which is a LIM-domain protein (see <PDOC00382>). - *Plasmodium falciparum* serine-

repeat protein (SERA), the major blood stage antigen. This protein of 111 Kd possesses a C-terminal thiol-protease-like domain [6], but the active site cysteine is replaced by a serine. The sequences around the three active site residues are well conserved and can be used as signature patterns.

5

Consensus pattern: Q-x(3)-[GE]-x-C-[YW]-x(2)-[STAGC]-[STAGCV] [C is the active site residue]- Note: the residue in position 4 of the pattern is almost always cysteine; the only exceptions are calpains (Leu), bleomycin hydrolase (Ser) and yeast YCP1 (Ser). -Note: the residue in position 5 of the pattern is always Gly except in papaya protease IV where it is

10 Glu.

Consensus pattern: [LIVMGSTAN]-x-H-[GSACE]-[LIVM]-x-[LIVMAT](2)-G-x-[GSADNH] [H is the active site residue]-

Consensus pattern: [FYCH]-[WI]-[LIVT]-x-[KRQAG]-N-[ST]-W-x(3)-[FYW]-G-x(2)-G-[LFYW]-[LIVMFYG]-x-[LIVMF] [N is the active site residue] - Note: these proteins belong to family C1 (papain-type) and C2 (calpains) in the classification of peptidases [7,11].-

15

[1] Dufour E. Biochimie 70:1335-1342(1988).[2] Kirschke H., Barrett A.J., Rawlings N.D. Protein Prof. 2:1587-1643(1995).[3] Shi G.-P., Chapman H.A., Bhairi S.M., Deleuw C., Reddy V.Y., Weiss S.J. FEBS Lett. 357:129-134(1995).[4] Velasco G., Ferrando A.A., Puente X.S., Sanchez L.M., Lopez-Otin C. J. Biol. Chem. 269:27136-27142(1994).[5] Chapot-Chartier M.P., Nardi M., Chopin M.C., Chopin A., Gripon J.C. Appl. Environ. Microbiol. 59:330-333(1993).[6] Higgins D.G., McConnell D.J., Sharp P.M. Nature 340:604-604(1989).[7] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:461-486(1994).

20

25 450. (peptidase M24) Aminopeptidase P and proline dipeptidase signature (1). Aminopeptidase P (EC 3.4.11.9) is the enzyme responsible for the release of any N-terminal amino acid adjacent to a proline residue. Proline dipeptidase (EC 3.4.13.9) (prolidase) splits dipeptides with a prolyl residue in the carboxyl terminal position. Bacterial aminopeptidase P 30 II (gene pepP) [1], proline dipeptidase (gene pepQ)[2], and human proline dipeptidase (gene PEPPD) [3] are evolutionary related. These proteins are manganese metalloenzymes. Yeast hypothetical proteins YER078c and YFR006w and Mycobacterium tuberculosis hypothetical protein MtCY49.29c also belong to this family. As a signature pattern for these enzymes a conserved region that contains three histidine residues has been developed

Consensus pattern: [HA]-[GSYR]-[LIVMT]-[SG]-H-x-[LIV]-G-[LIVM]-x-[IV]-H-[DE]-

[1] Yoshimoto T., Tone H., Honda T., Osatomi K., Kobayashi R., Tsuru D. J. Biochem.

- 5 105:412-416(1989).[2] Nakahigashi K., Inokuchi H. Nucleic Acids Res. 18:6439-6439(1990).[3] Endo F., Tanoue A., Nakai H., Hata A., Indo Y., Titani K., Matsuda I. J. Biol. Chem. 264:4476-4481(1989).[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

- 10 Methionine aminopeptidase signatures. (2). Methionine aminopeptidase (EC 3.4.11.18) (MAP) is responsible for the removal of the amino-terminal (initiator) methionine from nascent eukaryotic cytosolic and cytoplasmic prokaryotic proteins if the penultimate amino acid is small and uncharged. All MAP studied to date are monomeric proteins that require cobalt ions for activity. Two subfamilies of MAP enzymes are known to exist [1,2]. While
15 being evolutionary related, they only share a limited amount of sequence similarity mostly clustered around the residues shown, in the Escherichia coli MAP [3], to be involved in cobalt-binding. The first family consists of enzymes from prokaryotes as well as eukaryotic MAP-1, while the second group is made up of archebacterial MAP and eukaryotic MAP-2. The second subfamily also includes proteins which do not seem to be
20 MAP, but that are clearly evolutionary related such as mouse proliferation-associated protein 1 and fission yeast curved DNA-binding protein. For each of these subfamilies, a specific signature pattern that includes residues known to be involved in cobalt-binding has been developed.

- 25 Consensus pattern: [MFY]-x-G-H-G-[LIVMC]-[GSH]-x(3)-H-x(4)-[LIVM]-x-[HN]- [YWV] [H is a cobalt ligand]-
Consensus pattern: [DA]-[LIVMY]-x-K-[LIVM]-D-x-G-x-[HQ]-[LIVM]-[DNS]-G-x(3)-[DN] [The second D and the last D/N are cobalt ligands]

- 30 [1] Arfin S.M., Kendall R.L., Hall L., Weaver L.H., Stewart A.E., Matthews B.W., Bradshaw R.A. Proc. Natl. Acad. Sci. U.S.A. 92:7714-7718(1995).[2] Keeling P.J., Doolittle W.F. Trends Biochem. Sci. 21:285-286(1996).[3] Roderick S.L., Mathews B.W. Biochemistry 32:3907-3912(1993).[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

451. Cytochrome P450 cysteine heme-iron ligand signature

Cytochrome P450's [1,2,3,4] are a group of enzymes involved in the oxidative metabolism of a high number of natural compounds (such as steroids, fatty acids, prostaglandins, leukotrienes, etc) as well as drugs, carcinogens and mutagens. Based on sequence similarities, P450's have been classified into about forty different families [4,5]. P450's are proteins of 400 to 530 amino acids; the only exception is *Bacillus BM-3* (CYP102) which is a protein of 1048 residues that contains a N-terminal P450 domain followed by a reductase domain. P450's are heme proteins. A conserved cysteine residue in the C-terminal part of P450's is involved in binding the heme iron in the fifth coordination site. From a region around this residue, a ten residue signature was developed specific to P450's.

Consensus pattern: [FW]-[SGNH]-x-[GD]-x-[RHPT]-x-C-[LIVMFAP]-[GAD] [C is the heme iron ligand]-

[1] Nebert D.W., Gonzalez F.J. *Annu. Rev. Biochem.* 56:945-993(1987).

[2] Coon M.J., Ding X., Pernecky S.J., Vaz A.D.N. *FASEB J.* 6:669-673(1992).

[3] Guengerich F.P. *J. Biol. Chem.* 266:10019-10022(1991).

[4] Nelson D.R., Kamataki T., Waxman D.J., Guengerich F.P., Estrabrook R.W., Feyereisen R., Gonzalez F.J., Coon M.J., Gunsalus I.C., Gotoh O., Okuda K., Nebert D.W. *DNA Cell Biol.* 12:1-51(1993).

[5] Degtyarenko K.N., Archakov A.I. *FEBS Lett.* 332:1-8(1993).

452. (Pec Lyase) Pectate lyase

This enzyme forms a right handed beta helix structure. Pectate lyase is an enzyme involved in the maceration and soft rotting of plant tissue.

[1] Yoder MD, Keen NT, Jurnak F, *Science* 1993;260:1503-1507.

453. (pep M24) Aminopeptidase P and proline dipeptidase signature (pep1)

Aminopeptidase P (EC 3.4.11.9) is the enzyme responsible for the release of any N-terminal amino acid adjacent to a proline residue. Proline dipeptidase (EC 3.4.13.9) (prolidase) splits

dipeptides with a prolyl residue in the carboxyl terminal position. Bacterial aminopeptidase P II (gene pepP) [1], proline dipeptidase (gene pepQ)[2], and human proline dipeptidase (gene PEPPD) [3] are evolutionary related. These proteins are manganese metalloenzymes. Yeast hypothetical proteins YER078c and YFR006w and Mycobacterium tuberculosis .hypothetical protein MtCY49.29c also belong to this family. As a signature pattern for these enzymes a conserved region was selected that contains three histidine residues.

Consensus pattern: [HA]-[GSYR]-[LIVMT]-[SG]-H-x-[LIV]-G-[LIVM]-x-[IV]-H-[DE]-

[1] Yoshimoto T., Tone H., Honda T., Osatomi K., Kobayashi R., Tsuru D. J. Biochem. 105:412-416(1989).

[2] Nakahigashi K., Inokuchi H. Nucleic Acids Res. 18:6439-6439(1990).

[3] Endo F., Tanoue A., Nakai H., Hata A., Indo Y., Titani K., Matsuda I. J. Biol. Chem. 264:4476-4481(1989).

[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

Methionine aminopeptidase signatures (pep2)

Methionine aminopeptidase (EC 3.4.11.18) (MAP) is responsible for the removal of the amino-terminal (initiator) methionine from nascent eukaryotic cytosolic and cytoplasmic prokaryotic proteins if the penultimate amino acid is small and uncharged. All MAP studied to date are monomeric proteins that require cobalt ions for activity. Two subfamilies of MAP enzymes are known to exist [1,2]. While being evolutionary related, they only share a limited amount of sequence similarity mostly clustered around the residues shown, in the Escherichia coli MAP [3], to be involved in cobalt-binding. The first family consists of enzymes from prokaryotes as well as eukaryotic MAP-1, while the second group is made up of archeobacterial MAP and eukaryotic MAP-2. The second subfamily also includes proteins which do not seem to be MAP, but that are clearly evolutionary related such as mouse proliferation-associated protein 1 and fission yeast curved DNA-binding protein. For each of these subfamilies, a specific signature pattern was developed that includes residues known to be involved in cobalt-binding.

Consensus pattern: [MFY]-x-G-H-G-[LIVMC]-[GSH]-x(3)-H-x(4)-[LIVM]-x-[HN]-[YWV]
[H is a cobalt ligand]-

Consensus pattern: [DA]-[LIVMY]-x-K-[LIVM]-D-x-G-x-[HQ]-[LIVM]-[DNS]-G-x(3)-[DN] [The second D and the last D/N are cobalt ligands]

- [1] Arfin S.M., Kendall R.L., Hall L., Weaver L.H., Stewart A.E., Matthews B.W., Bradshaw R.A. Proc. Natl. Acad. Sci. U.S.A. 92:7714-7718(1995).
- [2] Keeling P.J., Doolittle W.F. Trends Biochem. Sci. 21:285-286(1996).
- [3] Roderick S.L., Mathews B.W. Biochemistry 32:3907-3912(1993).
- [4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

454. Peroxidases signatures

Peroxidases (EC 1.11.1.-) [1] are heme-binding enzymes that carry out a variety of biosynthetic and degradative functions using hydrogen peroxide as the electron acceptor. Peroxidases are widely distributed throughout bacteria, fungi, plants, and vertebrates. In peroxidases the heme prosthetic group is protoporphyrin IX and the fifth ligand of the heme iron is a histidine (known as the proximal histidine). Another histidine residue (the distal histidine) serves as an acid-base catalyst in the reaction between hydrogen peroxide and the enzyme. The regions around these two active site residues are more or less conserved in a majority of peroxidases [2,3]. The enzymes in which one or both of these regions can be found are listed below. - Yeast cytochrome c peroxidase (EC 1.11.1.5). - Myeloperoxidase (EC 1.11.1.7) (MPO). MPO is found in granulocytes and monocytes and plays a major role in the oxygen-dependent microbicidal system of neutrophils. - Lactoperoxidase (EC 1.11.1.7) (LPO). LPO is a milk protein which acts as an antimicrobial agent. - Eosinophil peroxidase (EC 1.11.1.7) (EPO). An enzyme found in the cytoplasmic granules of eosinophils. - Thyroid peroxidase (EC 1.11.1.8) (TPO). TPO plays a central role in the biosynthesis of thyroid hormones. It catalyzes the iodination and coupling of the hormonogenic tyrosines in thyroglobulin to yield the thyroid hormones T3 and T4. - Fungal ligninases. Ligninase catalyzes the first step in the degradation of lignin. It depolymerizes lignin by catalyzing the C(alpha)-C(beta) cleavage of the propyl side chains of lignin. - Plant peroxidases (EC 1.11.1.7). Plants express a large numbers of isozymes of peroxidases. Some of them play a role in cell-suberization by catalyzing the deposition of the aromatic residues of suberin on the cell wall, some are expressed as a defense response toward wounding, others are involved in the metabolism of auxin and the biosynthesis of lignin. - Prokaryotic catalase-peroxidases. Some bacterial species produce enzymes that exhibit both catalase and broad-spectrum

peroxidase activities [4]. Examples of such enzymes are: catalase HP I from *Escherichia coli* (gene katG) and perA from *Bacillus stearothermophilus*.

Consensus pattern: [DET]-[LIVMTA]-x(2)-[LIVM]-[LIVMSTAG]-[SAG]-[LIVMSTAG]-H-

5 [STA]-[LIVMFY] [H is the proximal heme-binding ligand] -

Consensus pattern: [SGATV]-x(3)-[LIVMA]-R-[LIVMA]-x-[FW]-H-x-[SAC] [H is an active site residue]-

[1] Dawson J.H. Science 240:433-439(1988).

10 [2] Kimura S., Ikeda-Saito M. Proteins 3:113-120(1988).

[3] Henrissat B., Saloheimo M., Lavaitte S., Knowles J.K.C. Proteins 8:251-257(1990).

[4] Welinder K.G. Biochim. Biophys. Acta 1080:215-220(1991).

15 455. pfkB family of carbohydrate kinases signatures

It has been shown [1,2,3] that the following carbohydrate and purine kinases are evolutionary related and can be grouped into a single family, which is known [1] as the 'pfkB family': -

Fructokinase (EC 2.7.1.4) (gene scrK). - 6-phosphofructokinase isozyme 2 (EC 2.7.1.11) (phosphofructokinase-2) (gene pfkB). pfkB is a minor phosphofructokinase isozyme in

20 *Escherichia coli* and is not evolutionarily related to the major isozyme (gene pfkA). Plants 6-phosphofructokinase also belong to this family. - Ribokinase (EC 2.7.1.15) (gene rbsK). -

Adenosine kinase (EC 2.7.1.20) (gene ADK). - 2-dehydro-3-deoxygluconokinase (EC 2.7.1.45) (gene: kdgK). - 1-phosphofructokinase (EC 2.7.1.56) (fructose 1-phosphate kinase) (gene fruK). - Inosine-guanosine kinase (EC 2.7.1.73) (gene gsk). - Tagatose-6-phosphate

25 kinase (EC 2.7.1.144) (phosphotagatokinase) (gene lacC). - *Escherichia coli* hypothetical protein yeiC. - *Escherichia coli* hypothetical protein yeiI. - *Escherichia coli* hypothetical protein yhfQ. - *Escherichia coli* hypothetical protein yihV. - *Bacillus subtilis* hypothetical protein yxdC. - Yeast hypothetical protein YJR105W. All the above kinases are proteins of

from 280 to 430 amino acid residues that share a few region of sequence similarity. Two of

30 these regions were selected as signature patterns. The first pattern is based on a region rich in glycine which is located in the N-terminal section of these enzymes; while the second pattern is based on a conserved region in the C-terminal section.

Consensus pattern: [AG]-G-x(0,1)-[GAP]-x-N-x-[STA]-x(6)-[GS]-x(9)-G-

Consensus pattern: [DNSK]-[PSTV]-x-[SAG](2)-[GD]-D-x(3)-[SAGV]-[AG]-[LIVMFYA]-[LIVMSTAP]

[1] Wu L.-F., Reizer A., Reizer J., Cai B., Tomich J.M., Saier M.H. Jr. *J. Bacteriol.*

5 173:3117-3127(1991).

[2] Orchard L.M.D., Kornberg H.L. *Proc. R. Soc. Lond., B, Biol. Sci.* 242:87-90(1990).

[3] Blatch G.L., Scholle R.R., Woods D.R. *Gene* 95:17-23(1990).

10 456. Phospholipase A2 active sites signatures

Phospholipase A2 (EC 3.1.1.4) (PA2) [1,2] is an enzyme which releases fatty acids from the second carbon group of glycerol. PA2's are small and rigid proteins of 120 amino-acid residues that have four to seven disulfide bonds. PA2 binds a calcium ion which is required for activity. The side chains of two conserved residues, a histidine and an aspartic acid, participate in a 'catalytic network'. Many PA2's have been sequenced from snakes, lizards, bees and mammals. In the latter, there are at least four forms: pancreatic, membrane-associated as well as two less characterized forms. The venom of most snakes contains multiple forms of PA2. Some of them are presynaptic neurotoxins which inhibit neuromuscular transmission by blocking acetylcholine release from the nerve termini. Two different signature patterns were derived for PA2's. The first is centered on the active site histidine and contains three cysteines involved in disulfide bonds. The second is centered on the active site aspartic acid and also contains three cysteines involved in disulfide bonds.

Consensus pattern: C-C-x(2)-H-x(2)-C [H is the active site residue] This pattern will not detect some snake toxins homologous with PA2 but which have lost their catalytic activity as well as otoconin-22, a *Xenopus* protein from the aragonitic otoconia which is also unlikely to be enzymatically active.

Consensus pattern: [LIVMA]-C-{LIVMFYWPCST}-C-D-x(5)-C [D is the active site residue] The majority of functional and non-functional PA2's. Undetected sequences are bee PA2, gila monster PA2's, PA2 PL-X from habu and PA2 PA-5 from mulga.

[1] Davidson F.F., Dennis E.A. *J. Mol. Evol.* 31:228-238(1990).

[2] Gomez F., Vandermeers A., Vandermeers-Piret M.-C., Herzog R., Rathe J., Stievenart M., Winand J., Christophe J. *Eur. J. Biochem.* 186:23-33(1989).

457. Phosphorylase pyridoxal-phosphate attachment site. Phosphorylases (EC 2.4.1.1) [1] are important allosteric enzymes in carbohydrate metabolism. They catalyze the formation of glucose 1-phosphate from polyglucose such as glycogen, starch or maltodextrin. Enzymes from different sources differ in their regulatory mechanisms and their natural substrates. However, all known phosphorylases share catalytic and structural properties. They are pyridoxal-phosphate dependent enzymes; the pyridoxal-P group is attached to a lysine residue around which the sequence is highly conserved and can be used as a signature pattern to detect this class of enzymes.

Consensus pattern: E-A-[SC]-G-x-[GS]-x-M-K-x(2)-[LM]-N [K is the pyridoxal-P attachment site]-

[1] Fukui T., Shimomura S., Nakano K. Mol. Cell. Biochem. 42:129-144(1982).

458. Protein kinases signatures and profile

Eukaryotic protein kinases [1 to 5] are enzymes that belong to a very extensive family of proteins which share a conserved catalytic core common to both serine/threonine and tyrosine protein kinases. There are a number of conserved regions in the catalytic domain of protein kinases. Two of these regions were selected to build signature patterns. The first region, which is located in the N-terminal extremity of the catalytic domain, is a glycine-rich stretch of residues in the vicinity of a lysine residue, which has been shown to be involved in ATP binding. The second region, which is located in the central part of the catalytic domain, contains a conserved aspartic acid residue which is important for the catalytic activity of the enzyme [6]; Two signature patterns were derived for that region: one specific for serine/threonine kinases and the other for tyrosine kinases. A profile was also developed which is based on the alignment in [1] and covers the entire catalytic domain.

Consensus pattern: [LIV]-G-{P}-G-{P}-[FYWMGSTNH]-[SGA]-{PW}-[LIVCAT]-{PD}-x-[GSTACLIVMFY]-x(5,18)-[LIVMFYWCSTAR]-[AIVP]-[LIVMFAGCKR]-K [K binds ATP]. The majority of known protein kinases belong to the class detected by this pattern, but

it fails to find a number of them, especially viral kinases which are quite divergent in this region and are completely missed by this pattern.

Consensus pattern: [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3) [D is an active site residue]. Most serine/ threonine specific protein kinases belong to this class detected by the pattern with 10 exceptions (half of them viral kinases) and also Epstein-Barr virus BGLF4 and Drosophila ninaC which have respectively Ser and Arg instead of the conserved Lys and which are therefore detected by the tyrosine kinase specific pattern described below.

Consensus pattern: [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-[RSTAC]-x(2)-N-[LIVMFYC](3)

[D is an active site residue] ALL tyrosine specific protein kinases with the exception of human ERBB3 and mouse blk belong to this class detected by the pattern. This pattern will also detect most bacterial aminoglycoside phosphotransferases [8,9] and herpesviruses gangciclovir kinases [10]; which are proteins structurally and evolutionary related to protein kinases. This profile also detects receptor guanylate cyclases and 2-5A-dependent ribonucleases. Sequence similarities between these two families and the eukaryotic protein kinase family have been noticed before. It also detects Arabidopsis thaliana kinase- like protein TMKL1 which seems to have lost its catalytic activity. If a protein analyzed includes the two protein kinase signatures, the probability of it being a protein kinase is close to 100%. Eukaryotic-type protein kinases have also been found in prokaryotes such as Myxococcus xanthus [11] and Yersinia pseudotuberculosis.

[1] Hanks S.K., Hunter T. FASEB J. 9:576-596(1995).

[2] Hunter T. Meth. Enzymol. 200:3-37(1991).

[3] Hanks S.K., Quinn A.M. Meth. Enzymol. 200:38-62(1991).

[4] Hanks S.K. Curr. Opin. Struct. Biol. 1:369-383(1991).

[5] Hanks S.K., Quinn A.M., Hunter T. Science 241:42-52(1988).

[6] Knighton D.R., Zheng J., Ten Eyck L.F., Ashford V.A., Xuong N.-H., Taylor S.S., Sowadski J.M. Science 253:407-414(1991).

[7] Bairoch A., Claverie J.-M. Nature 331:22(1988).

[8] Benner S. Nature 329:21-21(1987).

[9] Kirby R. J. Mol. Evol. 30:489-492(1992).

[10] Littler E., Stuart A.D., Chee M.S. Nature 358:160-162(1992).

[11] Munoz-Dorado J., Inouye S., Inouye M. Cell 67:995-1006(1991).

Receptor tyrosine kinase class II signature

A number of growth factors stimulate mitogenesis by interacting with a family of cell surface receptors which possess an intrinsic, ligand-sensitive, protein tyrosine kinase activity [1].

These receptor tyrosine kinases (RTK) all share the same topology: an extracellular ligand-

5 binding domain, a single transmembrane region and a cytoplasmic kinase domain. However they can be classified into at least five groups. The prototype for class II RTK's is the insulin receptor, a heterotetramer of two alpha and two beta chains linked by disulfide bonds. The alpha and beta chains are cleavage products of a precursor molecule. The alpha chain contains the ligand binding site, the beta chain transverse the membrane and contains the

10 tyrosine protein kinase domain. The receptors currently known to belong to class II are: -

Insulin receptor from vertebrates. - Insulin growth factor I receptor from mammals. - Insulin receptor-related receptor (IRR), which is most probably a receptor for a peptide belonging to the insulin family. - Insects insulin-like receptors. - Molluscan insulin-related peptide(s) receptor (MIP-R). - Insulin-like peptide receptor from Branchiostoma lanceolatum. - The

15 Drosophila developmental protein sevenless, a putative receptor for positional information required for the formation of the R7 photoreceptor cells. - The trk family of receptors (NTRK1, NTRK2 and NTRK3), which are high affinity receptors for nerve growth factor and related neurotrophic factors (BDNF and NT-3). And the following uncharacterized receptors: - ROS. - LTK (TYK1). - EDDR1 (cak, TRKE, RTK6). - NTRK3 (Tyro10, TKT). - A sponge

20 putative receptor tyrosine kinase. While only the insulin and the insulin growth factor I receptors are known to exist in the tetrameric conformation specific to class II RTK's, all the above proteins share extensive homologies in their kinase domain, especially around the putative site of autophosphorylation. Hence, a signature pattern was developed for this class of RTK's, which includes the tyrosine residue, itself probably autophosphorylated.

25 Consensus pattern: [DN]-[LIV]-Y-x(3)-Y-Y-R [The second Y is the autophosphorylation site]

[1] Yarden Y., Ullrich A. Annu. Rev. Biochem. 57:443-478(1988).

Receptor tyrosine kinase class III signature

A number of growth factors stimulate mitogenesis by interacting with a family of cell surface receptors which possess an intrinsic, ligand-sensitive, protein tyrosine kinase activity [1].

These receptor tyrosine kinases (RTK) all share the same topology: an extracellular ligand-

binding domain, a single transmembrane region and a cytoplasmic kinase domain. However they can be classified into at least five groups. The class III RTK's are characterized by the presence of five to seven immunoglobulin-like domains [2] in their extracellular section. Their kinase domain differs from that of other RTK's by the insertion of a stretch of 70 to 100 hydrophilic residues in the middle of this domain. The receptors currently known to belong to class III are: - Platelet-derived growth factor receptor (PDGF-R). PDGF-R exists as a homo- or heterodimer of two related chains: alpha and beta [3]. - Macrophage colony stimulating factor receptor (CSF-1-R) (also known as the fms oncogene). - Stem cell factor (mast cell growth factor) receptor (also known as the kit oncogene). - Vascular endothelial growth factor (VEGF) receptors Flt-1 and Flk-1/KDR [4]. - Fl cytokine receptor Flk-2/Flt-3 [5]. - The putative receptor Flt-4 [7]. a signature pattern was developed for this class of RTK's which is based on a conserved region in the kinase domain.

Consensus pattern: G-x-H-x-N-[LIVM]-V-N-L-L-G-A-C-T-

- [1] Yarden Y., Ullrich A. *Annu. Rev. Biochem.* 57:443-478(1988).
- [2] Hunkapiller T., Hood L. *Adv. Immunol.* 44:1-63(1989).
- [3] Lee K.-H., Bowen-Pope D.F., Reed R.R. *Mol. Cell. Biol.* 10:2237-2246(1990).
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- [5] Lyman S.D., James L., Vanden Bos T., de Vries P., Brasel K., Gliniak B., Hollingsworth L.T., Picha K.S., McKenna H.J., Spletter R.R. *Cell* 75:1157-1167(1993).
- [6] Galland F., Karamysheva A., Pebusque M.J., Borg J.P., Rottapel R., Dubreuil P., Rosnet O., Birnbaum D. *Oncogene* 8:1233-1240(1993).

Receptor tyrosine kinase class V signatures

A number of growth factors stimulate mitogenesis by interacting with a family of cell surface receptors which possess an intrinsic, ligand-sensitive, protein tyrosine kinase activity [1]. These receptor tyrosine kinases (RTK) all share the same topology: an extracellular ligand-binding domain, a single transmembrane region and a cytoplasmic kinase domain. However they can be classified into at least five groups on the basis of sequence similarities. The extracellular domain of class V RTK's consist of a region of about 300 amino acids, amongst which 16 conserved cysteines probably involved in disulfide bonds; this region is followed by two copies of a fibronectin type III domain. The ligands for these receptors are proteins of

about 200 to 300 residues collectively known as Ephrins. The receptors currently known to belong to class V are [2,3,E1]: - EPHA1 (Eph-1; Esk). - EPHA2 (Eck; Mpk-5; Sek-2). - EPHA3 (Etk-1; Hek; Mek4; Tyro4; Rek4; Cek4). - EPHA4 (Sek; Hek8; Mpk-3; Cek8). - EPHA5 (Ehk-1; Hek7; Bsk; Cek7). - EPHA6 (Ehk-2). - EPHA7 (Ehk-3; Hek11; Mdk-1; Ebk). - EPHA8 (Eek). - EPHB1 (Eph-2; Elk; Net). - EPHB2 (Eph-3; Hek5; Drt; Erk; Nuk; Sek-3; Cek5; Qek5). - EPHB3 (Hek-2; Mdk-5). - EPHB4 (Htk; Mdk-2; Myk-1). - EPHB5 (Cek9). The EPHA subtype receptors bind to GPI-anchored ephrins while the EPHB subtype receptors bind to type-I membrane ephrins. Two signature patterns were developed for this class of RTK's, which each include some of the conserved cysteine residues.

Consensus pattern: F-x-[DN]-x-[GAW]-[GA]-C-[LIVM]-[SA]-[LIVM](2)-[SA]-[LV]-[KRHQ]-[LIVA]-x(3)-[KR]-C-[PSAW] [The two C's are probably involved in disulfide bonds]

Consensus pattern: C-x(2)-[DE]-G-[DEQ]-W-x(2,3)-[PAQ]-[LIVMT]-[GT]-x-C-x-C-x(2)-G-[HFY]-[EQ] [The three C's are probably involved in disulfide bonds]

[1] Yarden Y., Ullrich A. Annu. Rev. Biochem. 57:443-478(1988).

[2] Sajjadi F.G., Pasquale E.B., Subramani S. New Biol. 3:769-778(1991).

[3] Wicks I.P., Wilkinson D., Salvaris E., Boyd A.W. Proc. Natl. Acad. Sci. U.S.A. 89:1611-1615(1992).

459. Protein kinase C terminal domain

460. Plant thionins signature

Thionins are small, basic, plant proteins generally toxic to animal cells [1]. They seem to exert their toxic effect at the level of the cell membrane but their exact function is not known. They consist of a polypeptide chain of forty five to fifty amino acids with three to four internal disulfide bonds. They are found in seeds but also in the cell wall of leaves [2]. Thionins are processed from larger precursor proteins [3]. Crambin [4], a hydrophobic plant seed protein, also belongs to this family. The pattern to detect this family of proteins includes three of the six cysteine residues involved in disulfide bonds. +-----+ | +-----+-----+ ||| | xxCCxxxxxxxxxxCxxxxxxxxCxxxCxxCxxxxCxxxxxxxx

***** ||| +-----+ 'C': conserved cysteine involved in a disulfide bond.¹⁰⁹;
position of the pattern.

Consensus pattern: C-C-x(5)-R-x(2)-[FY]-x(2)-C [The three C's are involved in disulfide
bonds] The proteins from the gamma-thionin family are not related to the above proteins and
are described in a separate section.

[1] Vernon L.P., Evett G.E., Zeikus R.D., Gray W.R. Arch. Biochem. Biophys. 238:18-
29(1985).

[2] Bohlmann H., Clausen S., Behnke S., Giese H., Hiller C., Reimann-Phillip U., Schrader
G., Barkholt V., Apel K. EMBO J. 7:1559-1565(1988).

[3] Bohlmann H., Apel K. Mol. Gen. Genet. 207:446-454(1987).

[4] Teeter M.M., Mazer J.A., L'Italien J.J. Biochemistry 20:5437-5443(1981).

461. Polyprenyl synthetases signatures

A variety of isoprenoid compounds are synthesized by various organisms. For example in
eukaryotes the isoprenoid biosynthetic pathway is responsible for the synthesis of a variety of
end products including cholesterol, dolichol, ubiquinone or coenzyme Q. In bacteria this
pathway leads to the synthesis of isopentenyl tRNA, isoprenoid quinones, and sugar carrier
lipids. Among the enzymes that participate in that pathway, are a number of polyprenyl
synthetase enzymes which catalyze a 1'4-condensation between 5 carbon isoprene units.

Currently the sequence of some of these enzymes is known: - Eukaryotic farnesyl
pyrophosphate synthetase (FPP synthetase) (EC 2.5.1.1 / EC 2.5.1.10) which catalyzes the
sequential condensation of isopentenyl pyrophosphate (IPP) with dimethylallyl
pyrophosphate (DMAPP), and then with the resultant geranyl pyrophosphate to form farnesyl
pyrophosphate. FPP synthetase is a cytoplasmic dimeric enzyme. - Prokaryotic farnesyl
pyrophosphate synthetase (gene ispA). - Prokaryotic octaprenyl diphosphate synthase (gene
ispB). - Prokaryotic heptaprenyl diphosphate synthase (EC 2.5.1.30). - Eukaryotic
geranylgeranyl pyrophosphate synthetase (GGPP synthetase) (EC 2.5.1.1 / EC 2.5.1.10 / EC
2.5.1.29) which catalyzes the sequential addition of the three molecules of IPP onto DMAPP
to form geranylgeranyl pyrophosphate. In plants GGPP synthase is a chloroplast enzyme
involved in the biosynthesis of terpenoids; in fungi, such as *Neurospora crassa* (gene al-3),
this enzyme is involved in the biosynthesis of carotenoids. - Prokaryotic GGPP synthetase,

which are involved in the biosynthesis of carotenoids (gene crtE). Such an enzyme is also encoded in the cyanelle genome of *Cyanophora paradoxa*. - Eukaryotic hexaprenyl pyrophosphate synthetase, which is involved in the biosynthesis of coenzyme Q and which catalyzes the formation of all trans- polyprenyl pyrophosphates generally ranging in length of
 5 between 6 and 10 isoprene units depending on the species. HP synthetase is a mitochondrial membrane-associated enzyme. It has been shown [1 to 5] that all the above enzymes share some regions of sequence similarity. Two of these regions are rich in aspartic-acid residues and could be involved in the catalytic mechanism and/or the binding of the substrates. signature patterns were developed for both regions. Possible additional members of this
 10 family of proteins are: - *Bacillus subtilis* spore germination protein C3 (gene gerC3). Both proteins are most probably also enzymes involved in isoprenoid metabolism [6].

Consensus pattern: [LIVM](2)-x-D-D-x(2,4)-D-x(4)-R-R-[GH]-

Consensus pattern: [LIVMFY]-G-x(2)-[FYL]-Q-[LIVM]-x-D-D-[LIVMFY]-x-[DNG]

[1] Ashby M.N., Edwards P.A. J. Biol. Chem. 265:13157-13164(1990).

[2] Fujisaki S., Hara H., Nishimura Y., Horiuchi K., Nishino T. J. Biochem. 108:995-1000(1990).

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[6] Bairoch A. Unpublished observations (1993).

462. Potato inhibitor I family signature

The potato inhibitor I family is one of the numerous families of serine proteinase inhibitors. Members of this protein family are found in plants; in the seeds of barley or beans [1,2,3],
 30 and in potato or tomato leaves where they accumulate in response to mechanical damage [4,5]. An inhibitor belonging to this family is also found in leech [6]. It is interesting to note that, currently, this is the only proteinase inhibitor family to be found both in plant and animal kingdoms. Structurally these inhibitors are small (60 to 90 residues) and in contrast with other families of protease inhibitors, they lack disulfide bonds. They have a single inhibitory

site. The consensus pattern includes three out of the four residues conserved in all members of this family and is located in the N-terminal half.

Consensus pattern: [FYW]-P-[EQH]-[LIV](2)-G-x(2)-[STAGV]-x(2)-A- Barley subtilisin-chymotrypsin inhibitor-2b has Glu instead of Gly. There is a trypsin inhibitor from the cucurbitaceae *Momordica charantia* [7], which is said to belong to the potato inhibitor I family but which shows only a very weak similarity with the other members of this family.

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[2] Svendsen I., Boisen S., Hejgaard J. Carlsberg Res. Commun. 47:45-53(1982).

[3] Nozawa H., Yamagata H., Aizono Y., Yoshikawa M., Iwasaki T. J. Biochem. 106:1003-1008(1989).

[4] Cleveland T.E., Thornburg R.W., Ryan C.A. Plant Mol. Biol. 8:199-207(1987).

[5] Lee J.S., Brown W.E., Graham J.S., Pearce G., Fox E.A., Dreher T.W., Ahern K.G.,

Pearson G.D., Ryan C.A. Proc. Natl. Acad. Sci. U.S.A. 83:7277-7281(1986).

[6] Seemuller U., Eulitz M., Fritz H., Strobl A. Hoppe-Seyler's Z. Physiol. Chem. 361:1841-1846(1980).

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463. (pp binding) Phosphopantetheine attachment site

Phosphopantetheine (or pantetheine 4' phosphate) is the prosthetic group of acyl carrier proteins (ACP) in some multienzyme complexes where it serves as a 'swinging arm' for the attachment of activated fatty acid and amino-acid groups [1]. Phosphopantetheine is attached to a serine residue in these proteins [2]. ACP proteins or domains have been found in various enzyme systems which are listed below (references are only provided for recently determined sequences). - Fatty acid synthetase (FAS), which catalyzes the formation of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH. Bacterial and plant chloroplast FAS are composed of eight separate subunits which correspond to the different enzymatic activities; ACP is one of these polypeptides. Fungal FAS consists of two multifunctional proteins, FAS1 and FAS2; the ACP domain is located in the N-terminal section of FAS2. Vertebrate FAS consists of a single multifunctional enzyme; the ACP domain is located between the beta-ketoacyl reductase domain and the C-terminal thioesterase domain [3]. - Polyketide antibiotics synthase enzyme systems. Polyketides are secondary metabolites produced from

simple fatty acids, by microorganisms and plants. ACP is one of the polypeptidic components involved in the biosynthesis of *Streptomyces* polyketide antibiotics actinorhodin, curamycin, granatacin, monensin, oxytetracycline and tetracenomycin C. - *Bacillus subtilis* putative polyketide synthases pksK, pksL and pksM which respectively contain three, five and one

5 ACP domains. - The multifunctional 6-methylsalicylic acid synthase (MSAS) from *Penicillium patulum*. This is a multifunctional enzyme involved in the biosynthesis of a polyketide antibiotic and which contains an ACP domain in the C-terminal extremity. - Multifunctional mycocerosic acid synthase (gene mas) from *Mycobacterium bovis*. - Gramicidin S synthetase I (gene grsA) from *Bacillus brevis*. This enzyme catalyzes the first
10 step in the biosynthesis of the cyclic antibiotic gramicidin S. - Tyrocidine synthetase I (gene tycA) from *Bacillus brevis*. The reaction carried out by tycA is identical to that catalyzed by grsA - Gramicidin S synthetase II (gene grsB) from *Bacillus brevis*. This enzyme is a multifunctional protein that activates and polymerizes proline, valine, ornithine and leucine. GrsB contains four ACP domains. - Erythronolide synthase proteins 1, 2 and 3 from
15 *Saccharopolyspora erythraea* which is involved in the biosynthesis of the polyketide antibiotic erythromycin. Each of these proteins contain two ACP domains. - Conidial green pigment synthase from *Aspergillus nidulans*. - ACV synthetase from various fungi. This enzyme catalyzes the first step in the biosynthesis of penicillin and cephalosporin. It contains three ACP domains. - Enterobactin synthetase component F (gene entF) from *Escherichia*
20 *coli*. This enzyme is involved in the ATP-dependent activation of serine during enterobactin (enterochelin) biosynthesis. - Cyclic peptide antibiotic surfactin synthase subunits 1, 2 and 3 from *Bacillus subtilis*. Subunits 1 and 2 contains three related domains while subunit 3 only contains a single domain. - HC-toxin synthetase (gene HTS1) from *Cochliobolus carbonum*. This enzyme synthesizes HC-toxin, a cyclic tetrapeptide. HTS1 contains four ACP domains. -
25 Fungal mitochondrial ACP [9], which is part of the respiratory chain NADH dehydrogenase (complex I). - *Rhizobium* nodulation protein nodF, which probably acts as an ACP in the synthesis of the nodulation Nod factor fatty acyl chain. The sequence around the phosphopantetheine attachment site is conserved in all these proteins and can be used as a signature pattern. A profile was also developed that spans the complete ACP-like domain.

30 Consensus pattern: [DEQGSTALMKRH]-[LIVMFYSTAC]-[GNQ]-[LIVMFYAG]-
[DNEKHS]-S- [LIVMST]-{PCFY}-[STAGCPQLIVMF]-[LIVMATN]-
[DENOGTAKRHL]- [LIVMWSTA]-[LIVGSTACR]-x(2)-[LIVMFA] [S is the pantetheine attachment site]

[1] Concise Encyclopedia Biochemistry, Second Edition, Walter de Gruyter, Berlin New-York (1988).

[2] Pugh E.L., Wakil S.J. J. Biol. Chem. 240:4727-4733(1965).

5 [3] Witkowski A., Rangan V.S., Randhawa Z.I., Amy C.M., Smith S. Eur. J. Biochem. 198:571-579(1991).

[6] Scotti C., Piatti M., Cuzzoni A., Perani P., Tognoni A., Grandi G., Galizzi A., Albertini A.M. Gene 130:65-71(1993).

10 [9] Sackmann U., Zensen R., Rohlen D., Jahnke U., Weiss H. Eur. J. Biochem. 200:463-469(1991).

464. (Prenyltrans) Terpene synthases signature

15 The following enzymes catalyze mechanistically related reactions which involve the highly complex cyclic rearrangement of squalene or its 2,3 oxide: - Lanosterol synthase (EC 5.4.99.7) (oxidosqualene--lanosterol cyclase), which catalyzes the cyclization of (S)-2,3-epoxysqualene to lanosterol, the initial precursor of cholesterol, steroid hormones and vitamin D in vertebrates and of ergosterol in fungi (gene ERG7). - Cycloartenol synthase (EC 5.4.99.8) (2,3-epoxysqualene--cycloartenol cyclase), a plant enzyme that catalyzes the
20 cyclization of (S)-2,3- epoxysqualene to cycloartenol. - Hopene synthase (EC 5.4.99.-) (squalene--hopene cyclase), a bacterial enzyme that catalyzes the cyclization of squalene into hopene, a key step in hopanoid (triterpenoid) metabolism. These enzymes are evolutionary related [1] proteins of about 70 to 85 Kd. As a signature pattern, a highly conserved region was selected which is rich in aromatic residues and which is located in the C-terminal section.

25 Consensus pattern: [DE]-G-S-W-x-G-x-W-[GA]-[LIVM]-x-[FY]-x-Y-[GA]

[1] Corey E.J., Matsuda S.P.T., Bartel B. Proc. Natl. Acad. Sci. U.S.A. 90:11628-11632(1993).

465. Prion protein signatures

Prion protein (PrP) [1,2,3] is a small glycoprotein found in high quantity in the brains of humans or animals infected with a number of degenerative neurological diseases such as

Kuru, Creutzfeldt-Jacob disease (CJD), scrapie or bovine spongiform encephalopathy (BSE). PrP is encoded in the host genome and expressed both in normal and infected cells. It has a tendency to aggregate yielding polymers called rods. Structurally, PrP is a protein consisting of a signal peptide, followed by an N-terminal domain that contains tandem repeats of a short motif (PHGGGWGQ in mammals, PHNPGY in chicken), itself followed by a highly conserved domain. It comes a C-terminal hydrophobic domain post-translationally removed when PrP is attached to the extracellular side of the cell membrane by a GPI-anchor. The structure of PrP is shown in the following schematic representation: +---+-----+
 *****-----+---+ [Sig] Tandem repeats | C C S | | +---+-----+---+
 10 -----|-----|---|+-----+ +-----+ | GPI'C': conserved cysteine involved in a disulfide bond.*: position of the patterns. As signature pattern for PrP, a perfectly conserved alanine- and glycine-rich region of 16 residues was selected as well as a region centered on the second cysteine involved in the disulfide bond.

15 Consensus pattern: A-G-A-A-A-G-A-V-V-G-G-L-G-G-Y-

Consensus pattern: E-x-[ED]-x-K-[LIVM](2)-x-[KR]-[LIVM](2)-x-[QE]-M-C-x(2)- Q-Y [C is involved in a disulfide bond]

[1] Stahl N., Prusiner S.B. FASEB J. 5:2799-2807(1991).

20 [2] Brunori M., Chiara Silvestrini M., Pocchiari M. Trends Biochem. Sci. 13:309-313(1988).

[3] Prusiner S.B. Annu. Rev. Microbiol. 43:345-374(1989).

466. Cyclophilin-type peptidyl-prolyl cis-trans isomerase signature and profile (pro
 25 isomerase)

Cyclophilin [1] is the major high-affinity binding protein in vertebrates for the immunosuppressive drug cyclosporin A (CSA). It exhibits a peptidyl- prolyl cis-trans isomerase activity (EC 5.2.1.8) (PPIase or rotamase). PPIase is an enzyme that accelerates protein folding by catalyzing the cis-trans isomerization of proline imidic peptide bonds in oligopeptides [2]. It is probable that CSA mediates some of its effects via an inhibitory action on PPIase. Cyclophilin is a cytosolic protein which belongs to a family [3,4,5] that also includes the following isozymes: - Cyclophilin B (or S-cyclophilin), a PPIase which is retained in an endoplasmic reticulum compartment. - Cyclophilin C, a cytoplasmic PPIase. - Mitochondrial matrix cyclophilin (cyp3). - A PPIase which seems specific for the folding of

rhodopsin and is an integral membrane protein anchored by a C-terminal transmembrane region. This protein was first characterized in *Drosophila* (gene *ninaA*). - Bacterial periplasmic PPIase (gene *ppiA*). - Bacterial cytosolic PPIase (gene *ppiB*). - Natural-killer cell cyclophilin-related protein. This large protein (about 160 Kd) is a component of a putative tumor-recognition complex involved in the function of NK cells. It contains a cyclophilin-type PPIase domain. - Mammalian nucleoporin Nup358 [6], a nuclear pore complex protein of 358 Kd that contains a C-terminal cyclophilin-type PPIase domain. - Yeast hypothetical protein YJR032w. - Fission yeast hypothetical protein SpAC21E11.05c. - *Caenorhabditis elegans* hypothetical protein T27D1.1. The sequences of the different forms of cyclophilin-type PPIases are well conserved. As a signature pattern, a conserved region was selected in the central part of these enzymes.

Consensus pattern: [FY]-x(2)-[STCNLV]-x-F-H-[RH]-[LIVMN]-[LIVM]-x(2)-F- [LIVM]-x-Q-[AG]-G- FKBP's, a family of proteins that bind the immunosuppressive drug FK506, are also PPIases, but their sequence is not at all related to that of cyclophilin.

[1] Stamnes M.A., Rutherford S.L., Zuker C.S. Trends Cell Biol. 2:272-276(1992).

[2] Fischer G., Schmid F.X. Biochemistry 29:2205-2212(1990).

[3] Trandinh C.C., Pao G.M., Saier M.H. Jr. FASEB J. 6:3410-3420(1992).

[4] Galat A. Eur. J. Biochem. 216:689-707(1993).

[5] Hacker J., Fischer G. Mol. Microbiol. 10:445-456(1993).

[6] Wu J., Matunis M.J., Kraemer D., Blobel G., Coutavas E. J. Biol. Chem. 270:14209-14213(1995).

467. Profilin signature

Profilin [1,2] is a small eukaryotic protein that binds to monomeric actin(G-actin) in a 1:1 ratio thus preventing the polymerization of actin into filaments (F-actin). It can also, in certain circumstance promotes actin polymerization. Profilin also binds to

polyphosphoinositides such as PIP2. Overall sequence similarity among profilin from organisms which belong to different phyla (ranging from fungi to mammals) is low, but the N-terminal region is relatively well conserved. That region is thought to be involved in the binding to actin. The signature pattern for profilin is based on conserved residues at the N-

terminal extremity. A protein structurally similar to profilin is present in the genome of variola and vaccinia viruses (gene A42R).

Consensus pattern: <x(0,1)-[STA]-x(0,1)-W-[DENQH]-x-[YI]-x-[DEQ]

5

[1] Haarer B.K., Brown S.S. Cell Motil. Cytoskeleton 17:71-74(1990).

[2] Sohn R.H., Goldschmidt-Clermont P. BioEssays 16:465-472(1994).

10 468. Protamine P1 signature

Protamines are small, highly basic proteins, that substitute for histones in sperm chromatin during the haploid phase of spermatogenesis. They pack sperm DNA into a highly condensed, stable and inactive complex. There are two different types of mammalian protamine, called P1 and P2. P1 has been found in all species studied, while P2 is sometimes absent. There seems to be a single type of avian protamine whose sequence is closely related to that of mammalian P1 [1]. As a signature for this family of proteins, a conserved region was selected at the N-terminal extremity of the sequence.

15

Consensus pattern: [AV]-R-[NFY]-R-x(2,3)-[ST]-x-S-x-S-

20

[1] Oliva R., Goren R., Dixon G.H. J. Biol. Chem. 264:17627-17630(1989).

469. Sperm histone P2 (protamine P2)

25

This protein also known as protamine P2 can substitute for histones in the chromatin of sperm. The alignment contains both the sequence of the mature P2 protein and its propeptide.

470. Proteasome A-type subunits signature

30

The proteasome (or macropain) (EC 3.4.99.46) [1 to 5, E1] is an eukaryotic and archaeobacterial multicatalytic proteinase complex that seems to be involved in an ATP/ubiquitin-dependent nonlysosomal proteolytic pathway. In eukaryotes the proteasome is composed of about 28 distinct subunits which form a highly ordered ring-shaped structure (20S ring) of about 700 Kd. Most proteasome subunits can be classified, on the basis on

sequence similarities into two groups, A and B. Subunits that belong to the A-type group are proteins of from 210 to 290 amino acids that share a number of conserved sequence regions. Subunits that are known to belong to this family are listed below. - Vertebrate subunits C2 (nu), C3, C8, C9, iota and zeta. - *Drosophila* PROS-25, PROS-28.1, PROS-29 and PROS-35. - Yeast C1 (PRS1), C5 (PRS3), C7-alpha (Y8) (PRS2), Y7, Y13, PRE5, PRE6 and PUP2. - *Arabidopsis thaliana* subunits alpha and PSM30. - *Thermoplasma acidophilum* alpha-subunit. In this archaeobacteria the proteasome is composed of only two different subunits. As a signature pattern for proteasome A-type subunits the best conserved region was selected, which is located in the N-terminal part of these proteins.

Consensus pattern: [FY]-x(4)-[STNV]-x-[FYW]-S-P-x-G-[RKH]-x(2)-Q-[LIVM]-[DE]-Y-[SAD]-x(2)-[SAG]-. These proteins belong to family T1 in the classification of peptidases [6,E2].

- [1] Rivett A.J. *Biochem. J.* 291:1-10(1993).
- [2] Rivett A.J. *Arch. Biochem. Biophys.* 268:1-8(1989).
- [3] Goldberg A.L., Rock K.L. *Nature* 357:375-379(1992).
- [4] Wilk S. *Enzyme Protein* 47:187-188(1993).
- [5] Hilt W., Wolf D.H. *Trends Biochem. Sci.* 21:96-102(1996).
- [6] Rawlings N.D., Barrett A.J. *Meth. Enzymol.* 244:19-61(1994).

Proteasome B-type subunits signature

The proteasome (or macropain) (EC 3.4.99.46) [1 to 5,E1] is an eukaryotic and archaeobacterial multicatalytic proteinase complex that seems to be involved in an ATP/ubiquitin-dependent nonlysosomal proteolytic pathway. In eukaryotes the proteasome is composed of about 28 distinct subunits which form a highly ordered ring-shaped structure (20S ring) of about 700 Kd. Most proteasome subunits can be classified, on the basis on sequence similarities into two groups, A and B. Subunits that belong to the B-type group are proteins of from 190 to 290 amino acids that share a number of conserved sequence regions. Subunits that are known to belong to this family are listed below. - Vertebrate subunits C5, beta, delta, epsilon, theta (C10-II), LMP2/RING12, C13 (LMP7/RING10), C7-I and MECL-1. - Yeast PRE1, PRE2 (PRG1), PRE3, PRE4, PRS3, PUP1 and PUP3. - *Drosophila* L(3)73A1. - Fission yeast pts1. - *Thermoplasma acidophilum* beta-subunit. In this archaeobacteria the proteasome is composed of only two different subunits. As a signature

pattern for proteasome B-type subunits the best conserved region was selected, which is located in the N-terminal part of these proteins.

Consensus pattern: [LIVMA]-[GSA]-[LIVMF]-x-[FYLVGAC]-x(2)-[GSACFY]-

- 5 [LIVMSTAC](3)-[GAC]-[GSTACV]-[DES]-x(15)-[RK]-x(12,13)-G-x(2)-[GSTA]-D-. These proteins belong to family T1 in the classification of peptidases [6,E2].

[1] Rivett A.J. Biochem. J. 291:1-10(1993).

[2] Rivett A.J. Arch. Biochem. Biophys. 268:1-8(1989).

- 10 [3] Goldberg A.L., Rock K.L. Nature 357:375-379(1992).

[4] Wilk S. Enzyme Protein 47:187-188(1993).

[5] Hilt W., Wolf D.H. Trends Biochem. Sci. 21:96-102(1996).

[6] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

15 471. (pyr redox) Pyridine nucleotide-disulphide oxidoreductases class-I active site

The pyridine nucleotide-disulphide oxidoreductases are FAD flavoproteins which contains a pair of redox-active cysteines involved in the transfer of reducing equivalents from the FAD cofactor to the substrate. On the basis of sequence and structural similarities [1] these

- 20 enzymes can be classified into two categories. The first category groups together the following enzymes [2 to 6]: - Glutathione reductase (EC 1.6.4.2) (GR). - Higher eukaryotes thioredoxin reductase (EC 1.6.4.5). - Trypanothione reductase (EC 1.6.4.8). - Lipoamide dehydrogenase (EC 1.8.1.4), the E3 component of alpha-ketoacid dehydrogenase complexes. - Mercuric reductase (EC 1.16.1.1). The sequence around the two cysteines involved in the
- 25 redox-active disulfide bond is conserved and can be used as a signature pattern.

Consensus pattern: G-G-x-C-[LIVA]-x(2)-G-C-[LIVM]-P [The two C's form the active site disulfide bond]. In positions 6 and 7 of the pattern all known sequences have Asn-(Val/ Ile) with the exception of GR from plant chloroplasts and from cyanobacteria which have Ile-Arg

30 [7].

[1] Kurlyan J., Krishna T.S.R., Wong L., Guenther B., Pahler A., Williams C.H. Jr., Model P. Nature 352:172-174(1991).

[2] Rice D.W., Schulz G.E., Guest J.R. J. Mol. Biol. 174:483-496(1984).

[3] Brown N.L. Trends Biochem. Sci. 10:400-402(1985).

[4] Carothers D.J., Pons G., Patel M.S. Arch. Biochem. Biophys. 268:409-425(1989).

[5] Walsh C.T., Bradley M., Nadeau K. Trends Biochem. Sci. 16:305-309(1991).

[6] Gasdaska P.Y., Gasdaska J.R., Cochran S., Powis G. FEBS Lett. 373:5-9(1995).

5 [7] Creissen G., Edwards E.A., Enard C., Wellburn A., Mullineaux P. Plant J. 2:129-131(1991).

472. (pyridoxal deC) DDC / GAD / HDC / TyrDC pyridoxal-phosphate attachment site
10 (pyridoxal deC)

Three different enzymes - all pyridoxal-dependent decarboxylases - seem to share regions of sequence similarity [1,2,3,4], especially in the vicinity of the lysine residue which serves as the attachment site for the pyridoxal-phosphate (PLP) group. These enzymes are: - Glutamate decarboxylase (EC 4.1.1.15) (GAD). Catalyzes the decarboxylation of glutamate into the
15 neurotransmitter GABA (4-aminobutanoate). - Histidine decarboxylase (EC 4.1.1.22) (HDC). Catalyzes the decarboxylation of histidine to histamine. There are two completely unrelated types of HDC: those that use PLP as a cofactor (found in Gram-negative bacteria and mammals), and those that contain a covalently bound pyruvoyl residue (found in Gram-positive bacteria). - Aromatic-L-amino-acid decarboxylase (EC 4.1.1.28) (DDC), also known
20 as L-dopa decarboxylase or tryptophan decarboxylase. DDC catalyzes the decarboxylation of tryptophan to tryptamine. It also acts on 5-hydroxy- tryptophan and dihydroxyphenylalanine (L-dopa). - Tyrosine decarboxylase (EC 4.1.1.25) (TyrDC) which converts tyrosine into tyramine, a precursor of isoquinoline alkaloids and various amides. These enzymes are collectively known as group II decarboxylases [3,4].

25 Consensus pattern: S-[LIVMFYW]-x(5)-K-[LIVMFYWG](2)-x(3)-[LIVMFYW]-x-[CA]-x(2)-[LIVMFYWQ]-x(2)-[RK] [K is the pyridoxal-P attachment site]

[1] Jackson F.R. J. Mol. Evol. 31:325-329(1990).

30 [2] Joseph D.R., Sullivan P., Wang Y.-M., Kozak C., Fenstermacher D.A., Behrendsen M.E., Zahnow C.A. Proc. Natl. Acad. Sci. U.S.A. 87:733-737(1990).

[3] Sandmeier E., Hale T.I., Christen P. Eur. J. Biochem. 221:997-1002(1994).

[4] Ishii S., Mizuguchi H., Nishino J., Hayashi H., Kagamiyama H. J. Biochem. 120:369-376(1996).

enzyme is unknown but it is likely to function in some aspects of cellular RNA processing. The reaction catalyzed by the enzyme occurs in three steps: 1) adenylation of the enzyme by ATP; 2) the enzyme acts on RNA-3'terminal phosphate to produce RNA-3'terminal diphosphate adenylate; 3) Release of AMP and cyclisation by a non catalytic nucleophilic attack by the adjacent 2'hydroxyl on the phosphorus in the diester linkage. This enzyme, which has been characterized in human (where there seems to be at least three isozymes) and *Escherichia coli* (gene *rtcA*), seems to be taxonomically widespread. It is found in insects, plants, fungi (gene *RTC1* in yeast) and in archaeobacteria. RNA cyclase is a protein of from 36 to 42 Kd. The best conserved region, which is used as a signature pattern, is a glycine-rich stretch of residues located in the central part of the sequence and which is reminiscent of various ATP, GTP or AMP glycine-rich loops. In this context, the conserved Arg (His in the *E.coli* enzyme) could be the AMP-binding residue.

Consensus pattern: [RH]-G-x(2)-P-x-G(3)-x-[LIV]-

[1] Genschik P., Billy E., Swianiewicz M., Filipowicz W. *EMBO J.* 16:2955-2967(1997).

[2] Filipowicz W., Vincente O. *Meth. Enzymol.* 181:499-510(1990).

475. REV protein (anti-repression trans-activator protein)

476. Prokaryotic-type class I peptide chain release factors signature (RF-I)

Peptide chain release factors (RFs) are required for the termination of protein biosynthesis

[1]. At present two classes of RFs can be distinguished. Class I RFs bind to ribosomes that have encountered a stop codon at their decoding site and induce release of the nascent polypeptide. Class II RFs are GTP-binding proteins that interact with class I RFs and enhance class I RF activity. In prokaryotes there are two class I RFs that act in a codon specific manner[2]: RF-1 (gene *prfA*) mediates UAA and UAG-dependent termination while RF-2(gene *prfB*) mediates UAA and UGA-dependent termination. RF-1 and RF-2 are structurally and evolutionary related proteins which have been shown [3] to make up a family that also contains the following proteins: - Fungal MRF1, a mitochondrial RF (m-RF) which recognizes the UAA and UAG codons. - *Escherichia coli* RF-H, a protein of unknown function. - *Escherichia coli* hypothetical protein *yaeJ* and a close *Pseudomonas putida*

homolog. A highly conserved region located in the central part of the 40 to 45 Kd RF-1/2 and m-RF and in the N-terminal of the 15 to 16Kd RF-H and yacJ is used as a signature pattern.

Consensus pattern: [AR]-[STA]-x-G-x-G-G-Q-[HNGCS]-V-N-x(3)-[ST]-A-[IV]

- 5 Note that prokaryotic-type class I RFs display no significant sequence similarity to prokaryotic-type class II which belong to the family of GTP-binding elongation factors nor to eukaryotic class I or class II RFs.

[1] Tate W.P. , Poole E.S., Mannering S.M. Prog. Nucleic Acids. Res. Mol. Biol. 52:293-335(1996).

[2] Craigen W.J., Lee C.C., Caskey C.T. Mol. Microbiol. 4:861-865(1990).

[3] Pel H.J., Rep M., Grivell L.A. Nucleic Acids Res. 20:4423-4428(1992).

15 477. RIO1/ZK632.3/MJ0444 family signature

The following uncharacterized proteins are evolutionary related [1]: - Yeast protein RIO1. - *Caenorhabditis elegans* hypothetical protein ZK632.3. - *Methanococcus jannaschii* hypothetical protein MJ0444. - *Thermoplasma acidophilum* hypothetical protein if rpoA2 3'region. The eukaryotic members of this family are proteins of about 55 to 60 Kd, while the archeobacterial ones are half that size. The central part of these proteins is highly conserved. The best conserved region is used as a signature pattern.

Consensus pattern: [LIVM]-V-H-[GA]-D-L-S-E-[FY]-N-x-[LIVM]

- 25 [1] Bairoch A. Unpublished observations (1997).

478. (RIP) Shiga/ricin ribosomal inactivating toxins active site signature. A number of bacterial and plant toxins act by inhibiting protein synthesis in eukaryotic cells. The toxins of the Shiga and ricin family inactivate 60S ribosomal subunits by an N-glycosidic cleavage which releases a specific adenine base from the sugar-phosphate backbone of 28S rRNA [1,2,3]. The toxins which are known to function in this manner are: - Shiga toxin from *Shigella dysenteriae* [4]. This toxin is composed of one copy of an enzymatically active A subunit and five copies of a B subunit responsible for binding the toxin complex to specific

receptors on the target cell surface. - Shiga-like toxins (SLT) are a group of *Escherichia coli* toxins very similar in their structure and properties to Shiga toxin. The sequence of two types of these toxins, SLT-1 [5] and SLT-2 [6], is known. - Ricin, a potent toxin from castor bean seeds. Ricin consists of two glycosylated chains linked by a disulfide bond. The A chain is enzymatically active. The B chain is a lectin with a binding preference for galactosides. Both chains are encoded by a single polypeptidic precursor. Ricin is classified as a type-II ribosome-inactivating protein (RIP); other members of this family are agglutinin, also from castor bean, and abrin from the seeds of the bean *Abrus precatorius* [7]. - Single chain ribosome-inactivating proteins (type-I RIP) from plants. Examples of such proteins are:

10 barley protein synthesis inhibitors I and II, mongolian snake-gourd trichosanthin, sponge gourd luffin-A and -B, garden four-o'clock MAP, common pokeberry PAP-S and soapwort saporin-6 [7]. All these toxins are structurally related. A conserved glutamic residue has been implicated [8] in the catalytic mechanism; it is located near a conserved arginine which also plays a role in catalysis [9]. The signature that has been developed for these proteins includes

15 these catalytic residues.

Consensus pattern: [LIVMA]-x-[LIVMSTA](2)-x-E-[SAGV]-[STAL]-R-[FY]-[RKNQS]-x-[LIVM]-[EQS]-x(2)-[LIVMF] [E and R are active site residues]-

20 [1] Endo Y., Tsurugi K., Takeda Y., Ogasawara T., Igarashi K. *Eur. J. Biochem.* 171:45-50(1988).[2] May M.J., Hartley M.R., Roberts L.M., Krieg P.A., Osborn R.W., Lord J.M. *EMBO J.* 8:301-308(1989).[3] Funatsu G., Islam M.R., Minami Y., Sung-Sil K., Kimura M. *Biochimie* 73:1157-1161(1991).[4] Strockbine N.A., Jackson M.P., Sung L.M., Holmes R.K., O'Brien A.D. *J. Bacteriol.* 170:1116-1122(1988).[5] Calderwood S.B., Auclair F.,

25 Donohue-Rolfe A., Keusch G.T., Mekalanos J.J. *Proc. Natl. Acad. Sci. U.S.A.* 84:4364-4368(1987).[6] Jackson M.P., Neill R.J., O'Brien A.D., Holmes R.K., Newland J.W. *FEMS Microbiol. Lett.* 44:109-114(1987).[7] Barbieri L., Battelli M.G., Stirpe F. *Biochim. Biophys. Acta* 1154:237-282(1993).[8] Hovde C.J., Calderwood S.B., Mekalanos J.J., Collier R.J. *Proc. Natl. Acad. Sci. U.S.A.* 85:2568-2572(1988).[9] Monzingo A.F., Collins

30 E.J., Ernst S.R., Irvin J.D., Robertus J.D. *J. Mol. Biol.* 233:705-715(1993).

479. Bacterial RNA polymerase, alpha chain (RNA pol A bac)

Members of this family include alpha subunit from eubacteria and alpha subunits from chloroplasts. The alpha subunit of RNA polymerase consists of two independently folded domains, referred to as amino-terminal and carboxyl terminal domains. The amino terminal domain is involved in the interaction with the other subunits of the RNA polymerase. The carboxyl-terminal domain interacts with the DNA and activators. The amino acid sequence of the alpha subunit is conserved in prokaryotic and chloroplast RNA polymerases. There are three regions of particularly strong conservation, two in the amino-terminal and one in the carboxyl-Comment: terminal [3].

[1] Zhang G, Darst SA; Science 1998;281:262-266. [2] Jeon YH, Negishi T, Shirakawa M, Yamazaki T, Fujita N, Ishihama A, Kyogoku Y; Science 1995;270:1495-1497. [3] Ebright RH, Busby S; Curr Opin Genet Dev 1995;5:197-203. [4] Murakami K, Kimura M, Owens JT, Meares CF, Ishihama A; Proc Natl Acad Sci USA 1997;94:1709-1714.

480. RNA polymerase beta subunit (RNA pol B)

RNA polymerases catalyse the DNA dependent polymerisation of RNA. Prokaryotes contain a single RNA polymerase compared to three in eukaryotes (not including mitochondrial and chloroplast polymerases). Each RNA polymerase complex contains two related members of this family, in each case they are the two largest subunits.

[1] Falkenburg D, Dworniczak B, Faust DM, Bautz EK; J Mol Biol 1987;195:929-937.

481. RNA polymerases H / 23 Kd subunits signature

In eukaryotes, there are three different forms of DNA-dependent RNA polymerases (EC 2.7.7.6) transcribing different sets of genes. Each class of RNA polymerase is an assemblage of ten to twelve different polypeptides. In archaeobacteria, there is generally a single form of RNA polymerase which also consist of an oligomeric assemblage of 10 to 13 polypeptides. Archaeobacterial subunit H (gene rpoH) [1,2] is a small protein of about 8.5 to 10 Kd, it is evolutionary related to the C-terminal part of a 23 Kd component shared by all three forms of eukaryotic RNA polymerases (gene RPB5 in yeast and POLR2E in mammals). As a signature pattern a conserved region was selected which is located at the N-terminal extremity of subunit H; this region contains two histidines that could play a role in the binding of a metal ion.

Consensus pattern: H-[NEI]-[LIVM]-V-P-x-H-x(2)-[LIVM]-x(2)-[DE]

[1] Klenk H.-P., Palm P., Lottspeich F., Zillig W. Proc. Natl. Acad. Sci. U.S.A. 89:407-410(1992).

- 5 [2] Thiru A., Hodach M., Eloranta J.J., Kostourou V., Weinzierl R.O., Matthews S.; J. Mol. Biol. 287:753-760(1999).

482. RNA polymerases K / 14 to 18 Kd subunits signature

- 10 In eukaryotes, there are three different forms of DNA-dependent RNA polymerases (EC 2.7.7.6) transcribing different sets of genes. Each class of RNA polymerase is an assemblage of ten to twelve different polypeptides. In archaeobacteria, there is generally a single form of RNA polymerase which also consist of an oligomeric assemblage of 10 to 13 polypeptides. A component of 14 to 18 Kd shared by all three forms of eukaryotic RNA polymerases and
- 15 which has been sequenced in budding yeast (gene RPB6 or RPO26), in fission yeast (gene rpb6 or rpo15), in human and in African swine fever virus [1] is evolutionary related [2] to archaeobacterial subunit K (gene rpoK). The archaeobacterial protein is colinear with the C-terminal part of the eukaryotic subunit.

20 Consensus pattern: [ST]-x-[FY]-E-x-[AT]-R-x-[LIVM]-[GSA]-x-R-[SA]-x-Q

[1] Lu Z., Kutish G.F., Sussman M.D., Rock D.L. Nucleic Acids Res. 21:2940-2940(1993).

[2] McKune K., Woychik N.A. J. Bacteriol. 176:4754-4756(1994).

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483. RNA polymerases L / 13 to 16 Kd subunits signature

- In eukaryotes, there are three different forms of DNA-dependent RNA polymerases (EC 2.7.7.6) transcribing different sets of genes. Each class of RNA polymerase is an assemblage of ten to twelve different polypeptides. In archaeobacteria, there is generally a single form of
- 30 RNA polymerase which also consist of an oligomeric assemblage of 10 to 13 polypeptides. It has been shown that small subunits of about 13 to 16 Kd found in all three types of eukaryotic polymerases are highly conserved. Subunits known to belong to this family are: - Budding yeast RPC19 subunit from RNA polymerases I and III [1]. - Budding yeast RPB11 subunit from RNA polymerase II [2]. - Mammalian RPB11 (gene POLR2K) from RNA

polymerase II. - *Caenorhabditis elegans* hypothetical protein F58A4.9. - *Methanococcus jannaschii* RNA polymerase subunit L (gene rpoL). - *Sulfolobus acidocaldarius* RNA polymerase subunit L (gene rpoL) [3]. As a signature pattern a conserved region was selected which is located at the N-terminal extremity of these polymerase subunits; this region contains two cysteines that could play a role in the binding of a metal ion.

Consensus pattern: [DE](2)-H-[ST]-[LIVM]-[GAP]-N-x(11)-V-x-[FM]-x(2)-Y-x(3)-H-P

[1] Dequard-Chablat M., Riva M., Carles C., Sentenac A. J. Biol. Chem. 266:15300-15307(1991).

[2] Woychik N.A., McKune K., Lane W.S., Young R.A. Gene Expr. 3:77-82(1993).

[3] Langer D. EMBL/GenBank: X70805.

484. RNA polymerases N / 8 Kd subunits signature

In eukaryotes, there are three different forms of DNA-dependent RNA polymerases (EC 2.7.7.6) transcribing different sets of genes. Each class of RNA polymerase is an assemblage of ten to twelve different polypeptides. In archaeobacteria, there is generally a single form of RNA polymerase which also consist of an oligomeric assemblage of 10 to 13 polypeptides.

Archaeobacterial subunit N (gene rpoN) [1] is a small protein of about 8 Kd, it is evolutionary related [2] to a 8.3 Kd component shared by all three forms of eukaryotic RNA polymerases (gene RPB10 in yeast and POLR2J in mammals) as well as to African swine fever virus protein CP80R [3]. As a signature pattern a conserved region was selected which is located at the N-terminal extremity of these polymerase subunits; this region contains two cysteines that could play a role in the binding of a metal ion.

Consensus pattern: [LIVMF](2)-P-[LIVM]-x-C-F-[ST]-C-G-

[1] Langer D., Hain J., Thuriaux P., Zillig W. Proc. Natl. Acad. Sci. U.S.A. 92:5768-5772(1995).

[2] McKune K., Woychik N.A. J. Bacteriol. 176:4754-4756(1994).

[3] Yanez R.J., Rodriguez J.M., Nogal M.L., Yuste L., Enriquez C., Rodriguez J.F., Vinuela E. Virology 208:249-278(1995).

485. Ribonuclease HII

[1] Mian IS; Nucleic Acids Res 1997;25:3187-3189.

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486. Ribonuclease PH signature

Prokaryotic ribonuclease PH (EC 2.7.7.56) (RNase PH) [1] is a phosphorolytic exoribonuclease that removes nucleotide residues following the -CCA terminus of tRNA and adds nucleotides to the ends of RNA molecules by using nucleoside diphosphates as substrates. RNase PH is a conserved protein of about 240 amino-acid residues. It is evolutionarily related to *Caenorhabditis elegans* hypothetical protein B0564.1. As a signature pattern, the most highly conserved region was selected which is located in the central part of these proteins.

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Consensus sequence: C-[DE]-[LIVM](2)-Q-[GTA]-D-G-[SG]-x(2)-[TA]-A

15

[1] Kelly K.O., Deutscher M.P. J. Biol. Chem. 267:17153-17158(1992).

487. RanBP1 domain

[1] Di Matteo G, Fuschi P, Zerfass K, Moretti S, Ricordy R, Cenciarelli C, Tripodi M,

20

Jansen-Durr P, Lavia P; Cell Growth Differ 1995;6:1213-1224.

488. Rhodanese signatures

Rhodanese (thiosulfate sulfurtransferase) (EC 2.8.1.1) [1,2] is an enzyme which catalyzes the transfer of the sulfane atom of thiosulfate to cyanide, to form sulfite and thiocyanate. In vertebrates, rhodanese is a mitochondrial enzyme of about 300 amino-acid residues involved in forming iron-sulfur complexes and cyanide detoxification. A cysteine residue takes part in the catalytic mechanism. Some bacterial proteins closely related to rhodanese are also thought to express a sulfotransferase activity. These are: - *Azotobacter vinelandii* rhdA. -

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- *Escherichia coli* sseA [3]. - *Saccharopolyspora erythraea* cysA [4]. - *Synechococcus* strain PCC 7942 rhdA [5]. RhdA is a periplasmic protein probably involved in the transport of sulfur compounds. Two patterns for the rhodanese family were developed. They are based on highly conserved regions, one which is located in the N-terminal region, the other at the C-terminal extremity of the enzyme.

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Consensus pattern: [FY]-x(3)-H-[LIV]-P-G-A-x(2)-[LIV]

Consensus pattern: [FY]-[DEAP]-G-[SA]-W-x-E-[FYW]

- 5 [1] Westley J. Meth. Enzymol. 77:285-291(1981).
 [2] Weiland K.L., Dooley T.P. Biochem. J. 275:227-231(1991).
 [3] Rudd K.E. Unpublished observations (1993).
 [4] Donadio S., Shafiee A., Hutchinson C.R. J. Bacteriol. 172:350-360(1990).
 [5] Laudenbach D.E., Ehrhardt D., Green L., Grossman A.R. J. Bacteriol. 173:2751-
 10 2760(1991).

489. Ribonuclease III family signature

- Prokaryotic ribonuclease III (EC 3.1.26.3) (gene rnc) [1] is an enzyme that digests double-
 15 stranded RNA. It is involved in the processing of ribosomal RNA precursors and of some
 mRNAs. RNase III is evolutionary related [2] to the following proteins: - Fission yeast *pac1*,
 a ribonuclease that probably inhibits mating and meiosis by degrading a specific mRNA
 required for sexual development. - Yeast ribonuclease III (gene RNT1), a dsRNA-specific
 nuclease that cleaves eukaryotic preribosomal RNA at various sites. - *Caenorhabditis elegans*
 20 hypothetical protein F26E4.13. - *Paramecium bursaria* chloroella virus 1 protein A464R. -
Synechocystis strain PCC 6803 hypothetical protein slr0346. - Fission yeast hypothetical
 protein SpAC8A4.08c, a protein with a N-terminal helicase domain and a C-terminal RNase
 III domain. - *Caenorhabditis elegans* hypothetical protein K12H4.8, a protein with the same
 structure as SpAC8A4.08c. These proteins share regions of sequence similarity; one of which
 25 is a highly conserved stretch of 9 residues which has been developed as a signature pattern.

Consensus pattern: [DEQ]-[RQ]-[LM]-E-[FYW]-[LV]-G-D-[SAR]-

- [1] Nashimoto H., Uchida H. Mol. Gen. Genet. 201:25-29(1985).
 30 [2] Mian I.S. Nucleic Acids Res. 25:3187-3195(1997).

490. Rieske iron-sulfur protein signatures

Ubiquinol-cytochrome c reductase (EC 1.10.2.2) (also known as the bc1 complex or complex III) is one of the electron transport chains of mitochondria and of some aerobic prokaryotes; it catalyzes the oxidoreduction of ubiquinol and cytochrome c. In the chloroplast of plants and in cyanobacteria plastoquinone-plastocyanin reductase (EC 1.10.99.1) (also known as the b6f complex) is functionally similar and catalyzes the oxidoreduction of plastoquinol and cytochrome f. One of the components of these electron transfer systems is an iron-sulfur protein with a 2Fe-2S cluster, which is called the Rieske protein [1,2]. The Rieske protein contains approximately 190 amino acid residues. The iron-sulfur cluster is complexed to the protein through cysteine and histidine residues. Two perfectly conserved regions in Rieske proteins contain all the residues that bind the iron-sulfur cluster. Both regions contain two cysteines and a histidine. The first cysteine and the histidine are 2Fe-2S ligands while the remaining cysteines form a disulfide bond [3]. Two conserved regions were selected as signature patterns.

Consensus pattern: C-[TK]-H-L-G-C-[LIVST] [The first C and the H are 2Fe-2S ligands] [The second C is involved in a disulfide bond]

Consensus pattern: C-P-C-H-x-[GSA] [The first C and the H are 2Fe-2S ligands] [The second C is involved in a disulfide bond]

[1] Gatti F.L., Meinhardt S.W., Ohnishi T., Tzagoloff A. J. Mol. Biol. 205:421-435(1989).

[2] Kallas T., Spiller S., Malkin R. Proc. Natl. Acad. Sci. U.S.A. 85:5794-5798(1988).

[3] Iwata S., Saynovits M., Link T.A., Michel H. Structure 4:567-579(1996).

491. Ribosomal protein L1 signature

Ribosomal protein L1 is the largest protein from the large ribosomal subunit. In *Escherichia coli*, L1 is known to bind to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1, 2], groups: - Eubacterial L1. - Algal and plant chloroplast L1. - Cyanelle L1. - Archaeobacterial L1. - Vertebrate L10A. - Yeast SSM1. As a signature pattern, the best conserved region was selected located in the central section of these proteins. It is located at the end of an alpha helix thought to be involved in RNA-binding.

Consensus pattern: [IM]-x(2)-[LIVA]-x(2,3)-[LIVM]-G-x(2)-[LMS]-[GSNH]-[PTKR]-
[KRAV]-G-x-[LIMF]-P-[DENSTKQ]

- [1] Nikonov S.V., Nevskaya N., Eliseikina I.A., Fomenkova N.P., Nikulin A., Ossina N.,
5 Garber M., Jonsson B.-H., Briand C., Al-Karadaghi S., Svensson L.A., Aevvarsson A., Liljas
A. EMBO J. 15:1350-1359(1996).
[2] Olvera J., Wool I.G. 2.3.CO;2-"Biochem. Biophys. Res. Commun. 220:954-957(1996).

10 492. Ribosomal protein L10 signature

Ribosomal protein L10 is one of the proteins from the large ribosomal subunit. L10 is a
protein of 162 to 185 amino-acid residues which has only been found so far in eubacteria. A
conserved region located in the N-terminal section of these proteins was used as a signature
pattern.

15 Consensus pattern: [DEH]-x(2)-[GS]-[LIVMF]-[STN]-[VA]-x-[DEQK]-[LIVMA]-x(2)-
[LIM]-R

20 493. Ribosomal protein L10e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis
of sequence similarities. One of these families consists of: - Vertebrate L10 (QM) [1]. - Plant
L10. - Caenorhabditis elegans L10 (F10B5.1). - Yeast L10 (QSR1). - Methanococcus
jannaschii MJ0543. These proteins have 174 to 232 amino-acid residues. A conserved region
25 located in the central section was selected as a signature pattern.

Consensus pattern: R-x-A-[FYW]-G-K-[PA]-x-G-x(2)-A-R-V

- [1] Chan Y.-L., Diaz J.-J., Denoroy L., Madjar J.-J., Wool I.G. 2.3.CO;2-"Biochem.
30 Biophys. Res. Commun. 255:952-956(1996).

494. Ribosomal protein L11 signature

Ribosomal protein L11 is one of the proteins from the large ribosomal subunit. In *Escherichia coli*, L11 is known to bind directly to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups:

- 5 - Eubacterial L11.
- Plant chloroplast L11 (nuclear-encoded).
- Red algal chloroplast L11.
- Cyanelle L11.
- Archaeobacterial L11.
- 10 - Mammalian L12.
- Plants L12.
- Yeast L12 (YL15).

L11 is a protein of 140 to 165 amino-acid residues. A conserved region located in the C-terminal section of these proteins was selected as a signature pattern. In *Escherichia coli*, the C-terminal half of L11 has been shown [3] to be in an extended and loosely folded conformation and is likely to be buried within the ribosomal structure.

Consensus pattern: [RKN]-x-[LIVM]-x-G-[ST]-x(2)-[SNQ]-[LIVM]-G-x(2)-[LIVM]-x(0,1)-
20 [DENG]

- [1] Pucciarelli G., Remacha M., Ballesta J.P.G.; *Nucleic Acids Res.* 18:4409-4416(1990).
- [2] Otake E., Hashimoto T., Mizuta K., Suzuki K.; *Protein Seq. Data Anal.* 5:301-313(1993).
- 25 [3] Choli T. *Biochem. Int.* 19:1323-1338(1989).

495. Ribosomal protein L7/L12 C-terminal domain

[1] Leijonmarck M, Liljas A; *J Mol Biol* 1987;195:555-579.

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496. Ribosomal protein L13 signature

Ribosomal protein L13 is one of the proteins from the large ribosomal subunit.

In *Escherichia coli*, L13 is known to be one of the early assembly proteins of

the 50S ribosomal subunit. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L13.

- Plant chloroplast L13 (nuclear-encoded). - Red algal chloroplast L13.

- Archaeobacterial L13. - Mammalian L13a (Tum P198). - Yeast Rp22 and Rp23.

- 5 L11 is a protein of 140 to 250 amino-acid residues. As a signature pattern, a conserved region was selected located in the C-terminal section of these proteins.

Consensus pattern: [LIVM]-[KRV]-[GK]-M-[LIV]-[PS]-x(4,5)-[GS]-[NOEKRA]-x(5)-
10 [LIVM]-x-[AIV]-[LFY]-x-[GDN]

[1] Chan Y.-L., Olvera J., Glueck A., Wool I.G. J. Biol. Chem. 269:5589-5594(1994).

15 497. Ribosomal protein L13e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities [1]. One of these families consists of:

- Vertebrate L13 (was previously known as Breast Basic Conserved protein 1 (BBC1)). - Drosophila L13. - Plant L13. - Yeast probable L13 (YM9375.11c).

- 20 These proteins have 199 to 218 amino-acid residues. As a signature pattern, a stretch of about 16 residues in the first third of these proteins selected.

-Consensus pattern: [KR]-Y-x(2)-K-[LIVM]-R-[STA]-G-[KR]-G-F-[ST]-L-x-E

- 25 [1] Olvera J., Wool I.G. Biochem. Biophys. Res. Commun. 201:102-107(1994).

498. Ribosomal protein L14 signature

Ribosomal protein L14 is one of the proteins from the large ribosomal subunit.

- 30 In eubacteria, L14 is known to bind directly to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L14. - Algal and plant chloroplast L14. - Cyanelle L14.

- Archaeobacterial L14. - Yeast L17A. - Mammalian L23.

- Caenorhabditis elegans L23 (B0336.10). - Higher eukaryotes mitochondrial L14.

- Yeast mitochondrial Yml38 (gene MRPL38).

L14 is a protein of 119 to 137 amino-acid residues. As a signature pattern, a conserved region located in the C-terminal half of these proteins was selected.

5 -Consensus pattern: [GA]-[LIV](3)-x(9,10)-[DNS]-G-x(4)-[FY]-x(2)-[NT]-x(2)-V-[LIV]

[1] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

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499. Ribosomal protein L15 signature

Ribosomal protein L15 is one of the proteins from the large ribosomal subunit. In *Escherichia coli*, L15 is known to bind the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1],

15 groups: - Eubacterial L15. - Plant chloroplast L15 (nuclear-encoded).
- Archaeobacterial L15. - Vertebrate L27a. - *Tetrahymena thermophila* L29.
- Fungi L27a (L29, CRP-1, CYH2).

L15 is a protein of 144 to 154 amino-acid residues. As a signature pattern, a conserved region was selected in the C-terminal section of these proteins.

20

-Consensus pattern: K-[LIVM](2)-[GASL]-x-[GT]-x-[LIVMA]-x(2,5)-[LIVM]-x-[LIVMF]-x(3,4)-[LIVMFCA]-[ST]-x(2)-A-x(3)-[LIVM]-x(3)-G

[1] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

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500. Ribosomal protein L15e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities [1]. One of these families consists of:

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- Mammalian L15. - Insect L15. - Plant L15. - Yeast YL10 (L13) (Rp15r).
- *Thermoplasma acidophilum* L15.

These proteins have about 200 amino acid residues. As a signature pattern, a conserved region was selected located in the central section.

-Consensus pattern: [DE]-[KR]-A-R-x-L-G-[FY]-x-[SAP]-x(2)-G-[LIVMFY](4)-R-x-R-[IV]-x-R-G

[1] Zwickl P., Lupas A., Baumeister W.

Biochem. Biophys. Res. Commun. 209:684-688(1995).

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501. Ribosomal protein L17 signature

Ribosomal protein L17 is one of the proteins from the large ribosomal subunit.

L17 belongs to a family of ribosomal proteins which, on the basis of sequence

10 similarities, groups: - Eubacterial L17.

- Yeast mitochondrial YmL8 (gene MRPL8).

Eubacterial L17 is a protein of 120 to 130 amino-acid residues. Yeast YmL8 is

twice larger (238 residues), the sequence of its N-terminal half is colinear

with that of eubacterial L17. As a signature pattern, a conserved region in

15 the N-terminal section was selected.

-Consensus pattern: I-x-[ST]-[GT]-x(2)-[KR]-x-K-x(6)-[DE]-x-[LIMV]-[LIVMT]-T-x-[STAG]-[KR]

20 502. Ribosomal protein L18e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrate L18 (known as L14 in *Xenopus*) [1]. - Plant L18.

- Yeast L18 (Rp28). - *Halobacterium marismortui* Hl29.

25 - *Sulfolobus acidocaldarius* Hl29e.

These proteins have 115 to 187 amino-acid residues., A stretch of about 13 residues in the first third of these proteins has been selected as a signature pattern.

-Consensus pattern: [KRE]-x-L-x(2)-[PS]-[KR]-x(2)-[RH]-[PSA]-x-[LIVM]-[NS]-[LIVM]-x-[RK]-[LIVM]

30 [1] Puder M., Barnard G.F., Staniunas R.J., Steele G.D. Jr., Chen L.B.

Biochim. Biophys. Acta 1216:134-136(1993).

503. Ribosomal L18p family

It has been shown that the amino terminal 93 amino acids of Swiss:P09895 are necessary and sufficient to bind 5S rRNA in vitro. The carboxyl-terminal half of the protein, comprising amino acids 151-296, serves to localize the protein to the nucleolus [1].

Number of members: 26

[1]

Medline: 96212235

Distinct domains in ribosomal protein L5 mediate 5 S rRNA binding and nucleolar localization.

Michael WM, Dreyfuss G;

J Biol Chem 1996;271:11571-11574.

504. Ribosomal protein L19 signature

Ribosomal protein L19 is one of the proteins from the large ribosomal subunit. In *Escherichia coli*, L19 is known to be located at the 30S-50S ribosomal subunit interface and may play a role in the structure and function of the aminoacyl-tRNA binding site. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups: - Eubacterial L19.

- Red algal chloroplast L19. - Cyanelle L19.

L19 is a protein of 120 to 130 amino-acid residues.,

A conserved region in the C-terminal section has been selected as a signature pattern.

-Consensus pattern: [LIVM]-x-[KRGTT]-x-[GSAI]-[KRQDA]-[VG]-[RSN]-X(0,1)-[KR]-[SA]-[KY]-[KLI]-[LYS]-Y-[LIM]-R

505. Ribosomal protein L19e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian ribosomal protein L19 [1]. - *Drosophila* ribosomal protein L19 [2].

- Slime mold (*D. discoideum*) vegetative specific protein V14 [3].

- Yeast ribosomal protein L19 (YL14). - Archeobacterial ribosomal protein L19E.

These proteins have 148 to 203 amino-acid residues.

A stretch of about 20 residues in the N-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: Q-[KR]-R-[LIVM]-x-[SA]-x(4)-[CV]-G-x(3)-[IV]-[WK]-[LIVF]-[DN]-P

- 5 [1] Chan Y.-L., Lin A., McNally J., Peleg D., Meyuhos O., Wool I.G.
J. Biol. Chem. 262:1111-1115(1987).[2] Hart K., Klein T., Wilcox M.
Mech. Dev. 43:101-110(1993).[3] Singleton C.K., Manning S.S., Ken R.
Nucleic Acids Res. 17:9679-9692(1989).

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506. Ribosomal protein L1e signature (Ribosomal_L4)

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists [1,2,3,4] of: - Vertebrate L1 (L4). - Drosophila L1. - Plant L1. - Yeast L2 (Rp2).

- 15 - Fission yeast L2. - Halobacterium marismortui HmaL4 (HL6).
- Methanococcus jannaschii MJ0177.

These proteins have 246 (archaeobacteria) to 427 (human) amino acids. A conserved region in the N-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: N-x(3)-[KRM]-x(2)-A-[LIVT]-x-S-A-[LIV]-x-A-[ST]-[SGA]-x(7)-[RK]-[GS]-H

- 20 [1] Rafti F., Gargiulo G., Manzi A., Malva C., Graziani F.
Nucleic Acids Res. 17:456-456(1989).[2] Presutti C., Villa T., Bozzoni I.
Nucleic Acids Res. 21:3900-3900(1993).
[3] Bagni C., Mariottini P., Annesi F., Amaldi F.
25 Biochim. Biophys. Acta 1216:475-478(1993).
[3] Arndt E., Kroemer W., Hatakeyama T. J. Biol. Chem. 265:3034-3039(1990).

507. Ribosomal protein L2 signature

- 30 Ribosomal protein L2 is one of the proteins from the large ribosomal subunit.
In Escherichia coli, L2 is known to bind to the 23S rRNA and to have peptidyltransferase activity. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial L2.
- Algal and plant chloroplast L2. - Cyanelle L2. - Archaeobacterial L2.

- Plant L2. - Slime mold L2. - Marchantia polymorpha mitochondrial L2.
- Paramecium tetraurelia mitochondrial L2. - Fission yeast K5, K37 and KD4.
- Yeast YL6. - Vertebrate L8.

The best conserved region located in the C-terminal section of these proteins has been

5 selected as

a signature pattern.

-Consensus pattern: P-x(2)-R-G-[STAIV](2)-x-N-[APK]-x-[DE]

[1] Marty I., Meyer Y.

Nucleic Acids Res. 20:1517-1522(1992).

10 [2] Otake E., Hashimoto T., Mizuta K., Suzuki K.

Protein Seq. Data Anal. 5:301-313(1993).

508. Ribosomal protein L20 signature

15 Ribosomal protein L20 is one of the proteins from the large ribosomal subunit.

In Escherichia coli, L20 is known to bind directly to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L20. - Algal and plant chloroplast L20.

- Cyanelle L20.

20 L20 is a protein of about 120 amino-acid residues. A conserved region located in the central section of these proteins has been selected as a signature pattern.

-Consensus pattern: K-x(3)-[KRC]-x-[LIVM]-W-[IV]-[STNALV]-R-[LIVM]-[NS]-x(3)-[RKHS]

[1] Otake E., Hashimoto T., Mizuta K., Suzuki K.

25 Protein Seq. Data Anal. 5:301-313(1993).

509. Ribosomal protein L21e signature

30 A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L21 [1]. - Entamoeba histolytica L21 [2].

- Caenorhabditis elegans L21 (C14B9.7). - Yeast L21E (URP1) [3].

- Halobacterium marismortui HL31 [4].

These proteins have 160 (eukaryotes) or 95 (archeobacteria) amino-acid

residues. A conserved region in the central part of these proteins has been selected as a signature pattern.

-Consensus pattern: G-[DE]-x-V-x(10)-[GV]-x(2)-[FYH]-x(2)-[FY]-x-G-x-T-G

[1] Devi K.R.G., Chan Y.-L., Wool I.G.

5 Biochem. Biophys. Res. Commun. 162:364-370(1989).

[2] Petter R., Rozenblatt S., Nuchamowitz Y., Mirelman D.

Mol. Biochem. Parasitol. 56:329-333(1992).

[3] Jank B., Waldherr M., Schweyen R.J. Curr. Genet. 23:15-18(1993).

[4] Hatakeyama T., Kimura M. Eur. J. Biochem. 172:703-711(1988).

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510. Ribosomal protein L21 signature

Ribosomal protein L21 is one of the proteins from the large ribosomal subunit.

In *Escherichia coli*, L21 is known to bind to the 23S rRNA in the presence of

15 L20. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups: - Eubacterial L21.

- *Marchantia polymorpha* chloroplast L21. - *Cyanelle* L21.

- *Spinach* chloroplast L21 (nuclear-encoded).

Eubacterial L21 is a protein of about 100 amino-acid residues, the mature form

20 of the *spinach* chloroplast L21 has 200 residues. A conserved region located in the C-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: [IVT]-x(3)-[KR]-x(3)-[KRO]-K-x(6)-G-[HF]-R-[RO]-x(2)-[ST]

25 511. Ribosomal protein L22 signature

Ribosomal protein L22 is one of the proteins from the large ribosomal subunit.

In *Escherichia coli*, L22 is known to bind 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3], groups: - Eubacterial L22.

30 - Algal and plant chloroplast L22 (in legumes L22 is encoded in the nucleus instead of the chloroplast). - *Cyanelle* L22. - Archaeobacterial L22.

- Mammalian L17. - Plant L17. - Yeast YL17.

A conserved region located in the C-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: [RKQN]-x(4)-[RH]-[GAS]-x-G-[KRQS]-x(9)-[HDN]-[LIVM]-x-[LIVMS]-x-[LIVM]

[1] Gantt J.S., Baldauf S.L., Calie P.J., Weeden N.F., Palmer J.D.

EMBO J. 10:3073-3078(1991), [2] Madsen L.H., Kreiberg J.D., Gausing K.

5 Curr. Genet. 19:417-422(1991).

[3] Otaka E., Hashimoto T., Mizuta K., Suzuki K.

Protein Seq. Data Anal. 5:301-313(1993).

10 512. Ribosomal protein L23 signature

Ribosomal protein L23 is one of the proteins from the large ribosomal subunit.

In *Escherichia coli*, L23 is known to bind a specific region on the 23S rRNA; in yeast, the corresponding protein binds to a homologous site on the 26S rRNA [1]. It belongs to a family of ribosomal proteins which, on the basis of

15 sequence similarities [2,3,4], groups: - Eubacterial L23.

- Algal and plant chloroplast L23. - Archaeobacterial L23. - Mammalian L23A.

- *Caenorhabditis elegans* L23A (F55D10.2). - Fungi L25.

- Yeast mitochondrial YmL41 (gene MRPL41 or MRP20).

20 A small conserved region in the C-terminal section of these proteins, which is probably involved in rRNA-binding has been selected as a signature pattern [2].

-Consensus pattern: [RK](2)-[AM]-[IVFYT]-[IV]-[RKT]-L-[STANEOK]-x(7)-[LIVMFT]

[1] El Baradi T.T.A.L., Raue H.A., van de Regt C.H.F., Verbree E.C.,

Planta R.J. EMBO J. 4:210-2107(1985).

25 [2] Raue H.A., Otaka E., Suzuki K. J. Mol. Evol. 28:418-426(1989).

[3] Fearon K., Mason T.L. J. Biol. Chem. 267:5162-5170(1992).

[4] Otaka E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

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513. Ribosomal protein L24 signature

Ribosomal protein L24 is one of the proteins from the large ribosomal subunit.

L24 belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups: - Eubacterial L24.

- Plant chloroplast L24 (nuclear-encoded). - Red algal L24. - Vertebrate L26.

- Yeast L26 (YL33). - Archaeobacterial HmaL24 (HL15).

- A probable ribosomal protein from *Sulfolobus acidocaldarius* [1].

In their mature form, these proteins have 103 to 150 amino-acid residues.

- 5 A conserved stretch of 20 residues in their N-terminal section has been selected as a signature pattern.

-Consensus pattern: [GDEN]-D-x-V-x-[IV]-[LIVMA]-x-G-x(2)-[KRA]-[GNO]-x(2,3)-[GA]-x-[IV]

[1] Ouzounis C., Kyriakides N., Sander C.

- 10 Nucleic Acids Res. 23:565-570(1995).

514. Ribosomal protein L24e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped

- 15 on the basis of sequence similarities. One of these families consists [1] of:

- Mammalian ribosomal protein L24.

- Yeast ribosomal protein L30A/B (Rp29) (YL21).

- *Kluyveromyces lactis* ribosomal protein L30.

- *Arabidopsis thaliana* ribosomal protein L24 homolog.

- 20 - *Haloarcula marismortui* ribosomal protein HL21/HL22.

- *Methanococcus jannaschii* MJ1201.

These proteins have 60 to 160 amino-acid residues. The most conserved region, which is located in the N-terminal region of these proteins has been selected as a signature pattern.

-Consensus pattern: [FY]-x-[GSH]-x(2)-[IV]-x-P-G-x-G-x(2)-[FYV]-x-[KRHE]-x-D

- 25 [1] Chan Y.-L., Olvera J., Wool I.G.

Biochem. Biophys. Res. Commun. 202:1176-1180(1994).

515. Ribosomal protein L27 signature

- 30 Ribosomal protein L27 is one of the proteins from the large ribosomal subunit.

L27 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial L27.

- Plant chloroplast L27 (nuclear-encoded). - Algal chloroplast L27.

- Yeast mitochondrial YmL2 (gene MRPL2 or MRP7).

The schematic relationship between these groups of proteins is shown below.

Eub. L27 NxxxxxxxxAlgal L27 Nxxxxxxxx

Plant L27 ttttNxxxxxxxxxxxx

Yeast MRP7 tttNxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

5 ***t': transit peptide.

'N': N-terminal of mature protein. '*': position of the pattern.

-Consensus pattern: G-x-[LIVM](2)-x-R-Q-R-G-x(5)-G

[1] Elhag G.A., Bourque D.P. Biochemistry 31:6856-6864(1992).

[2] Otake E., Hashimoto T., Mizuta K.

10 Protein Seq. Data Anal. 5:285-300(1993).

516. Ribosomal L28 family

15 The ribosomal 28 family includes L28 proteins from bacteria and chloroplasts. The L24 protein from yeast Swiss:P36525 also contains a region of similarity to prokaryotic L28 proteins. L24 from yeast is also found in the large ribosomal subunit

Number of members: 24

517. Ribosomal protein L29 signature

Ribosomal protein L29 is one of the proteins from the large ribosomal subunit.

25 L29 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L29. - Red algal L29.

- Archaeobacterial L29. - Mammalian L35 - Caenorhabditis elegans L35 (ZK652.4).

- Yeast L35.

L29 is a protein of 63 to 138 amino-acid residues.

30 A conserved region located in the central section of L29 has been selected as a signature pattern.

-Consensus pattern: [KNQS]-[PSTL]-x(2)-[LIMFA]-[KRGSA]-x-[LIVYSTA]-[KR]-[KRHQS]-[DESTANRL]-[LIV]-A-[KRCQVT]-[LIVMA]

[1] Otake E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

518. Ribosomal protein L3 signature

Ribosomal protein L3 is one of the proteins from the large ribosomal subunit.

- 5 In *Escherichia coli*, L3 is known to bind to the 23S rRNA and may participate in the formation of the peptidyltransferase center of the ribosome. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3,4], groups: - Eubacterial L3. - Red algal L3. - Cyanelle L3. - Archaeobacterial *Halobacterium marismortui* HmaL3 (HL1).
- 10 - Yeast L3 (also known as trichodermin resistance protein) (gene TCM1). - *Arabidopsis thaliana* L3 (genes ARP1 and ARP2). - Mammalian L3 (L4). - Mammalian mitochondrial L3. - Yeast mitochondrial YmL9 (gene MRPL9).
- A conserved region located in the central section of these proteins has been selected as a signature pattern.
- 15 - Consensus pattern: [FL]-x(6)-[DN]-x(2)-[AGS]-x-[ST]-x-G-[KRH]-G-x(2)-G-x(3)-R
 [1] Arndt E., Kroemer W., Hatakeyama T. J. Biol. Chem. 265:3034-3039(1990).
 [2] Graack H.-R., Grohmann L., Kitakawa M., Schaefer K.L., Kruft V.
 Eur. J. Biochem. 206:373-380(1992).
 [3] Herwig S., Kruft V., Wittmann-Liebold B.
 20 Eur. J. Biochem. 207:877-885(1992).
 [4] Otake E., Hashimoto T., Mizuta K., Suzuki K.
 Protein Seq. Data Anal. 5:301-313(1993).

25 519. Ribosomal protein L30 signature

Ribosomal protein L30 is one of the proteins from the large ribosomal subunit.

L30 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L30. - Archaeobacterial L30.

- *Drosophila* L7. - Slime mold L7. - Mammalian L7. - Fungi L7 (YL8).
- 30 - Yeast mitochondrial L33.
- L30 from eubacteria are small proteins of about 60 residues, those from archaeobacteria are proteins of about 150 residues. Eukaryotic L7 are proteins of about 250 to 270 residues. The schematic relationship between the three groups of proteins is shown below. Eub. L30 NxxxxxxxxxC

Arc. L30 NxxxxxxxxxxxxxxxxxxxxxxxxxxxxC

Euk. L7 NxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxC

*****1*: position of the pattern.

The signature pattern for this family of ribosomal proteins spans the

- 5 N-terminal half of the region common to all these proteins.

-Consensus pattern: [IVT]-[LIVM]-x(2)-[LF]-x-[LI]-x-[KRHQEG]-x(2)-[STNQH]-x-
[IVT]-x(10)-[LMS]-[LIV]-x(2)-[LIVA]-x(2)-[LMFY]-[IVT]

[1] Mizuta K., Hashimoto T., Otaka E.

Nucleic Acids Res. 20:1011-1016(1992).

10

520. Ribosomal protein L31 signature

Ribosomal protein L31 is one of the proteins from the large ribosomal subunit.

L31 is a protein of 66 to 97 amino-acid residues which has only been found so
15 far in eubacteria and in some algal chloroplasts.

A conserved region located in the central section of these proteins has been selected as
a signature pattern.

-Consensus pattern: H-P-F-[FY]-[TI]-x(9)-G-R-[AIV]-x-[KRQ]

20

521. Ribosomal protein L31e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped
on the basis of sequence similarities. One of these families consists of:

- Mammalian L31 [1]. - Chlamydomonas reinhardtii L31. - Yeast L34.

- 25 - Halobacterium marismortui HL30 [2].

These proteins have 87 to 128 amino-acid residues.

A conserved region, located in the central section has been selected as a signature pattern.

-Consensus pattern: V-[KR]-[LIVM]-x(3)-[LIVM]-N-x-[AKH]-x-W-x-[KR]-G

[1] Tanaka T., Kuwano Y., Kuzumaki T., Ishikawa K., Ogata K.

- 30 Eur. J. Biochem. 162:45-48(1987).[2] Bergmann U., Arndt E.

Biochim. Biophys. Acta 1050:56-60(1990).

522. Ribosomal protein L33 signature

Ribosomal protein L33 is one of the proteins from the large ribosomal subunit. In *Escherichia coli*, L33 has been shown to be on the surface of 50S subunit. L33 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3], groups: - Eubacterial L33.

- 5 - Algal and plant chloroplast L33. - Cyanelle L33.

L33 is a small protein of 49 to 66 amino-acid residues. A conserved region located in the central section of L33 has been selected as a signature pattern.

-Consensus pattern: Y-x-[ST]-x-[KR]-[NS]-x(4)-[PATQ]-x(1,2)-[LIVM]-[EA]-x(2)-K-[FY]-[CSD]

- 10 [1] Kruft V., Kapp U., Wittmann-Liebold B. *Biochimie* 73:855-860(1991).

[2] Sharp P.M. *Gene* 139:129-130(1994).

[3] Otake E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

15

523. Ribosomal protein L34 signature

Ribosomal protein L34 is one of the proteins from the large subunit of the prokaryotic ribosome. It is a small basic protein of 44 to 51 amino-acid residues [1]. L34 belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups: -

- 20 Eubacterial L34.

- Red algal chloroplast L34. - Cyanelle L34.

A conserved region that corresponds to the N-terminal half of L34 has been selected as a signature pattern.

-Consensus pattern: K-[RG]-T-[FYWL]-[EQS]-x(5)-[KRHS]-x(4,5)-G-F-x(2)-R

- 25 [1] Old I.G., Margarita D., Saint Girons I.

Nucleic Acids Res. 20:6097-6097(1992).

524. Ribosomal protein L34e signature

- 30 A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L34. - Mosquito L31 [1]. - Plant L34 [2].

- Yeast putative ribosomal protein YIL052c. - *Methanococcus jannaschii* MJ0655.

These proteins have 89 to 129 amino-acid residues.

A conserved region located in the N-terminal section of these proteins has been

selected as a signature pattern.

-Consensus pattern: Y-x-[ST]-x-S-[NY]-x(5)-[KR]-T-P-G

[1] Lan Q., Niu L.L., Fallon A.M.

Biochim. Biophys. Acta 1218:460-462(1994).

5 [2] Gao J., Kim S.R., Chung Y.Y., Lee J.M., An G.

Plant Mol. Biol. 25:761-770(1994).

525. Ribosomal protein L35Ae signature

10 A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrate L35A. - *Caenorhabditis elegans* L35A (F10E7.7).

- Yeast L37A/L37B (Rp47). - *Pyrococcus woesei* L35A homolog [1].

These proteins have 87 to 110 amino-acid residues.

15 A highly conserved stretch of 22 residues in the C-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: G-K-[LIVM]-x-R-x-H-G-x(2)-G-x-V-x-A-x-F-x(3)-[LI]-P

[1] Ouzounis C., Kyriades N., Sander C.

Nucleic Acids Res. 23:565-570(1995).

526. Ribosomal protein L36 signature

Ribosomal protein L36 is the smallest protein from the large subunit of the prokaryotic ribosome. It belongs to a family of ribosomal proteins which, on the basis of sequence

25 similarities [1], groups: - Eubacterial L36. - Algal and plant chloroplast L36. - Cyanelle

L36.L36 is a small basic and cysteine-rich protein of 37 amino-acid residues. As a signature pattern, a conserved region that corresponds to positions 11 to 36 in L36 and includes three conserved cysteine residues has been developed.

Consensus pattern: C-x(2)-C-x(2)-[LIVM]-x-R-x(3)-[LIVMN]-x-[LIVM]-x-C-x(3,4)-[KR]-

30 H-x-Q-x-Q-

[1] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

527. Ribosomal protein L36e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of: - Mammalian L36 [1].

- Drosophila L36 (M(1)1B). - Caenorhabditis elegans L36 (F37C12.4).

- Candida albicans L39. - Yeast YL39.

- 5 These proteins have 99 to 104 amino acids.

A conserved region in the central part of these proteins has been selected as a signature pattern.

-Consensus pattern: P-Y-E-[KR]-R-x-[LIVM]-[DE]-[LIVM](2)-[KR]

[1] Chan Y.-L., Paz V., Olvera J., Wool I.G.

- 10 Biochem. Biophys. Res. Commun. 192:849-853(1993).

528. Ribosomal protein L39e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L39 [1]. - Plants L39. - Yeast L46 [2]. - Archeobacterial L39e [3].

These proteins are very basic. About 50 residues long, they are the smallest proteins of eukaryotic-type ribosomes. A conserved region in the C-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: [KRA]-T-x(3)-[LIVM]-[KQF]-x-[NHS]-x(3)-R-[NHY]-W-R-R

[1] Lin A., McNally J., Wool I.G. J. Biol. Chem. 259:487-490(1984).

[2] Leer R.J., van Raamsdonk-Duin M.M.C., Kraakman P., Mager W.H., Planta R.J. Nucleic Acids Res. 13:701-709(1985).

[3] Ramirez C., Louie K.A., Matheson A.T. FEBS Lett. 250:416-418(1989).

529. Ribosomal L40e family

Bovine L40 has been identified as a secondary RNA binding protein [1]. L40 is fused to a ubiquitin protein [2].

- 30 Number of members: 27

[1]

Medline: 88203200

RNA binding proteins of the large subunit of bovine mitochondrial ribosomes.

Piatyszek MA, Denslow ND, O'Brien TW;

Nucleic Acids Res 1988;16:2565-2583.

[2]Medline: 96011832

The carboxyl extensions of two rat ubiquitin fusion proteins

5 are ribosomal proteins S27a and L40.

Chan YL, Suzuki K, Wool IG;

Biochem Biophys Res Commun 1995;215:682-690.

10 530. (Ribosomal L44) Ribosomal protein L44e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L44 [1]. - Trypanosoma brucei L44.

- Caenorhabditis elegans L44 (C09H10.2). - Fungal L44 (L41).

15 - Halobacterium marismortui LA [2].

These proteins have 92 to 105 amino-acid residues.

A conserved region located in the C-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: K-x-[TV]-K-K-x(2)-L-[KR]-x(2)-C

20 [1] Gallagher M.J., Chan Y.-L., Lin A., Wool I.G. DNA 7:269-273(1988).

[2] Bergmann U., Wittmann-Liebold B.

Biochim. Biophys. Acta 1173:195-200(1993)

25 531. Ribosomal protein L5 signature

Ribosomal protein L5 is one of the proteins from the large ribosomal subunit.

In Escherichia coli, L5 is known to be involved in binding 5S RNA to the large ribosomal subunit. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3,4], groups: - Eubacterial L5.

30 - Algal chloroplast L5. - Cyanelle L5. - Archaeobacterial L5. - Mammalian L11.

- Tetrahymena thermophila L21. - Slime mold L5 (V18). - Yeast L16 (39A).

- Plants mitochondrial L5.

L5 is a protein of about 180 amino-acid residues.

A conserved region, located in the first third of these

proteins has been selected as a signature pattern.

-Consensus pattern: [LIVM]-x(2)-[LIVM]-[STAVC]-[GE]-[QV]-x(2)-[LIVMA]-x-[STC]-x-[STAG]-[KRH]-x-[STA]

[1] Hatakeyama T., Hatakeyama T. Biochim. Biophys. Acta 1039:343-347(1990).

5 [2] Rosendahl G., Andreassen P.H., Kristiansen K. Gene 98:161-167(1991).

[3] Yang D., Gunther I., Matheson A.T., Auer J., Spicker G., Boeck A.

Biochimie 73:679-682(1991).

[4] Otaka E., Hashimoto T., Mizuta K., Suzuki K.

Protein Seq. Data Anal. 5:301-313(1993).

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532. ribosomal L5P family C-terminus

This region is found associated with Ribosomal_L5.

Number of members: 60

15

533. Ribosomal protein L6 signatures

Ribosomal protein L6 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L6 is known to bind directly to the 23S rRNA and is located at the aminoacyl-tRNA binding site of the peptidyltransferase center. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3,4], groups: - Eubacterial L6.

20

- Algal chloroplast L6.
- Cyanelle L6.
- 25 - Archaeobacterial L6.
- Marchantia polymorpha mitochondrial L6.
- Yeast mitochondrial YmL6 (gene MRPL6).
- Mammalian L9.
- Drosophila L9.
- 30 - Plants L9.
- Yeast L9 (YL11).

25

30

While all the above proteins are evolutionary related it is very difficult to derive a pattern that will find them all. Two patterns were therefore created, the first to detect

eubacterial, cyanelle and mitochondrial L6, the second to detect archaeobacterial L6 as well as eukaryotic L9.

-Consensus pattern: [PS]-[DENS]-x-Y-K-[GA]-K-G-[LIVM]

-Consensus pattern: Q-x(3)-[LIVM]-x(2)-[KR]-x(2)-R-x-F-x-D-G-[LIVM]-Y-[LIVM]-x(2)-

5 [KR]

[1] Suzuki K., Olvera J., Wool I.G. Gene 93:297-300(1990).

[2] Schwank S., Harrer R., Schueller H.-J., Schweizer E. Curr. Genet. 24:136-140(1993).

[3] Golden B.L., Ramakrishnan V., White S.W. EMBO J. 12:4901-4908(1993).

10 [4] Otake E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

534. Ribosomal protein L6e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian ribosomal protein L6 (L6 was previously known as TAX-responsive enhancer element binding protein 107).

- Caenorhabditis elegans ribosomal protein L6 (R151.3).

- Yeast ribosomal protein YL16A/YL16B.

- Mesembryanthemum crystallinum ribosomal protein YL16-like.

These proteins have 175 (yeast) to 287 (mammalian) amino acids. A highly conserved region in the central part of these proteins has been selected as a signature pattern.

-Consensus pattern: N-x(2)-P-L-R-R-x(4)-[FY]-V-I-A-T-S-x-K

535. Ribosomal protein L7Ae signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrate L7A (SURF3) [1]. - Plant L7A. - Yeast L7A (YL5) (Rp6).

- Yeast protein NHP2 [2]. - Yeast hypothetical protein YEL026w.

- Bacillus subtilis hypothetical protein ylxQ. - Halobacterium marismortui Hs6.

- Methanococcus jannaschii MJ1203.

These proteins have 100 to 265 amino-acid residues.

A conserved region located in the central section has been selected as a signature pattern.

-Consensus pattern: [CA]-x(4)-[IV]-P-[FY]-x(2)-[LIVM]-x-[GSQ]-[KRO]-x(2)-L-G

[1] Colombo P., Yon J., Garson K., Fried M.

Proc. Natl. Acad. Sci. U.S.A. 89:6358-6362(1992).

5 [2] Kolodrubetz D., Burgum A. Yeast 7:79-90(1991).

536. Ribosomal protein L9 signature

Ribosomal protein L9 is one of the proteins from the large ribosomal subunit.

10 In Escherichia coli, L9 is known to bind directly to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities

[1,2], groups: - Eubacterial L9. - Cyanobacterial L9.

- Plant chloroplast L9 (nuclear-encoded). - Red algal chloroplast L9.

A conserved region, located in the N-terminal section of these proteins has been selected
15 as a signature pattern.

-Consensus pattern: G-x(2)-[GN]-x(4)-V-x(2)-G-[FY]-x(2)-N-[FY]-L-x(5)-[GA]-
x(3)-[STN]

[1] Hoffman D.W., Davies C., Gerchman S.E., Kycia J.H., Porter S.J.,

White S.W., Ramakrishnan V. EMBO J. 13:205-212(1994).

20 [2] Otake E., Hashimoto T., Mizuta K., Suzuki K.

Protein Seq. Data Anal. 5:301-313(1993).

537. Ribosomal protein S10 signature

25 Ribosomal protein S10 is one of the proteins from the small ribosomal subunit.

In Escherichia coli, S10 is known to be involved in binding tRNA to the ribosomes. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial S10.

- Algal chloroplast S10. - Cyanelle S10. - Archaeobacterial S10.

30 - Marchantia polymorpha and Prototheca wickerhamii mitochondrial S10.

- Arabidopsis thaliana mitochondrial S10 (nuclear encoded). - Vertebrate S20.

- Plant S20. - Yeast URP2.

S10 is a protein of about 100 amino-acid residues.

A conserved region located in the center of these proteins has been selected as a signature pattern.

-Consensus pattern: [AV]-x(3)-[GDNSR]-[LIVMSTA]-x(3)-G-P-[LIVM]-x-[LIVM]-P-T
[1] Otaka E., Hashimoto T., Mizuta K.

5 Protein Seq. Data Anal. 5:285-300(1993).

538. Ribosomal protein S11 signature

Ribosomal protein S11 [1] plays an essential role in selecting the correct tRNA in protein biosynthesis. It is located on the large lobe of the small ribosomal subunit. S11 belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups [2]: - Eubacterial S11.

- Algal and plant chloroplast S11. - Cyanelle S11. - Archaeobacterial S11.

- Marchantia polymorpha and Prototheca wickerhamii mitochondrial S11.

15 - Acanthamoeba castellanii mitochondrial S11. - Neurospora crassa S14 (crp-2).

- Yeast S14 (RP59 or CRY1).

- Mammalian, Drosophila, Trypanosoma, and plant S14.

- Caenorhabditis elegans S14 (F37C12.9).

One of the best conserved regions in these proteins was selected as a signature pattern.

-Consensus pattern: [LIVMF]-x-[GSTAC]-[LIVMF]-x(2)-[GSTAL]-x(0,1)-[GSN]-
[LIVMF]-x-[LIVM]-x(4)-[DEN]-x-T-P-x-[PA]-[STCH]-[DN]

[1] Kimura M., Kimura J., Hatakeyama T. FEBS Lett. 240:15-20(1988).

[2] Otaka E., Hashimoto T., Mizuta K.

25 Protein Seq. Data Anal. 5:285-300(1993).

539. Ribosomal protein S12 signature

Ribosomal protein S12 is one of the proteins from the small ribosomal subunit.

30 In Escherichia coli, S12 is known to be involved in the translation initiation step. It is a very basic protein of 120 to 150 amino-acid residues. S12 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial S12. - Archaeobacterial S12.

- Algal and plant chloroplast S12. - Cyanelle S12.

- Protozoa and plant mitochondrial S12. - Yeast S28.

- Drosophila mitochondrial protein tko (Technical KnockOut). - Mammalian S23.

The best conserved regions in these proteins, located in the center of each sequence have been selected as a signature pattern.

- 5 -Consensus pattern: [RK]-x-P-N-S-[AR]-x-R

[1] Otake E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

- 10 540. Ribosomal protein S12e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of: - Vertebrate S12 [1].

- Trypanosoma brucei S12 [2]. - Caenorhabditis elegans S12 (F54E7.2).

- Drosophila S12. - Yeast S12.

- 15 These proteins have 130 to 150 amino acids.

A conserved region in the N-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: A-L-[KROP]-x-V-L-x(2)-[SA]-x(3)-[DN]-G-L

[1] Lin A., Chan Y.-L., Jones R., Wool I.G.

- 20 J. Biol. Chem. 262:14343-14351(1987).[2] Marchal C., Ismaili N., Pays E.

Mol. Biochem. Parasitol. 57:331-334(1993).

541. Ribosomal protein S13 signature

- 25 Ribosomal protein S13 is one of the proteins from the small ribosomal subunit.

In Escherichia coli, S13 is known to be involved in binding fMet-tRNA, and hence, in the initiation of translation. It is a basic protein of 115 to 177

amino-acid residues and belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S13.

- 30 - Plant chloroplast S13 (nuclear encoded). - Red algal chloroplast S13.

- Cyanelle S13. - Archaeobacterial S13. - Plant mitochondrial S13.

- Mammalian and plant S18.

The best conserved regions in these proteins, located in their C-terminal part have been selected as a signature pattern.

-Consensus pattern: [KRQS]-G-x-R-H-x(2)-[GSNH]-x(2)-[LIVMC]-R-G-Q

[1] Chan Y.-L., Paz V., Wool I.G.

Biochem. Biophys. Res. Commun. 178:1212-1218(1991).

[2] Otaka E., Hashimoto T., Mizuta K.

5 Protein Seq. Data Anal. 5:285-300(1993).

542. Ribosomal protein S14p/S29e (Ribosomal protein S14 signature)

Ribosomal protein S14 is one of the proteins from the small ribosomal subunit. In

10 Escherichia coli, S14 is known to be required for the assembly of 30S particles and may also be responsible for determining the conformation of 16S rRNA at the A site. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups:

- Eubacterial S14.
- Algal and plant chloroplast S14.
- 15 - Cyanelle S14.
- Archaeobacterial Methanococcus vannielii S14.
- Plant mitochondrial S14.
- Yeast mitochondrial MRP2.
- Mammalian S29.
- 20 - Yeast YS29A/B.

S14 is a protein of 53 to 115 amino-acid residues. Our signature pattern is based on the few conserved positions located in the center of these proteins.

Consensus pattern: [RP]-x(0,1)-C-x(11,12)-[LIVMF]-x-[LIVMF]-[SC]-[RG]-x(3)-[RN]

[1] Chan Y.-L., Suzuki K., Olvera J., Wool I.G. Nucleic Acids Res. 21:649-655(1993).

[2] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

30 543. Ribosomal protein S15 signature

Ribosomal protein S15 is one of the proteins from the small ribosomal subunit.

In Escherichia coli, this protein binds to 16S ribosomal RNA and functions at early steps in ribosome assembly. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S15.

- Archaeobacterial Halobacterium marismortui HmaS15 (HS11).
 - Plant chloroplast S15. - Yeast mitochondrial S28. - Mammalian S13.
 - Brugia pahangi and Wuchereria bancrofti S13 (S15). - Yeast S13 (YS15).
- S15 is a protein of 80 to 250 amino-acid residues.

5 A conserved region located in the C-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: [LIVM]-x(2)-H-[LIVMFY]-x(5)-D-x(2)-[SAGN]-x(3)-[LF]-x(9)-[LIVM]-x(2)-[FY]

[1] Dang H., Ellis S.R.

10 Nucleic Acids Res. 18:6895-6901(1990).

[2] Otake E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

15 544. Ribosomal protein S16 signature

Ribosomal protein S16 is one of the proteins from the small ribosomal subunit. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups to:

- Eubacterial S16.
- Algal and plant chloroplast S16.
- Cyanelle S16.
- Neurospora crassa mitochondrial S24 (cyt-21).

S16 is a protein of about 100 amino-acid residues. A conserved region located in the N-terminal extremity of these proteins has been selected as a signature pattern.

Consensus pattern: [LIVMT]-x-[LIVM]-[KR]-L-[STAK]-R-x-G-[AKR]

[1] Otake E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

545. Ribosomal protein S17 signature

Ribosomal protein S17 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S17 is known to bind specifically to the 5' end of 16S ribosomal RNA and is thought to be involved in the recognition of termination

codons. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3], groups: - Eubacterial S17.

- Plant chloroplast S17 (nuclear encoded). - Red algal chloroplast S17.

- Cyanelle S17. - Archaeobacterial S17. - Mammalian and plant cytoplasmic S11.

5 - Yeast S18a and S18b (RP41; YS12).

The best conserved regions located in the C-terminal sections of these proteins have been selected as a signature pattern.

-Consensus pattern: G-D-x-[LIV]-x-[LIVA]-x-[OEK]-x-[RK]-P-[LIV]-S

[1] Gantt J.S., Thompson M.D. J. Biol. Chem. 265:2763-2767(1990).

10 [2] Herfurth E., Hirano H., Wittmann-Liebold B.

Biol. Chem. Hoppe-Seyler 372:955-961(1991).

[3] Otake E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

15 546. Ribosomal protein S17e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrates S17 [1]. - Drosophila S17 [2]. - Neurospora crassa S17 (crp-3).

20 - Yeast S17a (RP51A) and S17b (RP51B) [3]. - Methanococcus jannaschii MJ0245.

These proteins have from 63 (in archebacteria) to 130 to 146 amino acids and are highly conserved. A region in the central part of these proteins has been selected as a signature.

-Consensus pattern: A-x-I-x-[ST]-K-x-L-R-N-[KR]-I-A-G-[FY]-x-T-H

25 [1] Chen I.-T., Roufa D.J. Gene 70:107-116(1988).

[2] Maki C., Rhoads D.D., Stewart M.J., van Slyke B., Denell R.E.,

Roufa D.J. Gene 79:289-298(1989).[3] Abovich N., Rosbash M.

Mol. Cell. Biol. 4:1871-1879(1984).

30 547. Ribosomal protein S18 signature

Ribosomal protein S18 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S18 has been involved in aminoacyl-tRNA binding[1]. It appears to be situated at the tRNA A-site of the ribosome. It belongs to a family of ribosomal proteins

which, on the basis of sequence similarities[2], groups: - Eubacterial S18. - Algal and plant chloroplast S18. - Cyanelle S18. As a signature pattern, a conserved region in the central section of the protein has been selected. This region contains two basic residues which may be involved in RNA-binding.-

- 5 Consensus pattern: [IV]-[DY]-Y-x(2)-[LIVMT]-x(2)-[LIVM]-x(2)-[FYT]-[LIVM]-[ST]-[DERP]-x-[GY]-K-[LIVM]-x(3)-R-[LIVMAS]-
 [1] McDougall J., Choli T., Kruff V., Kapp U., Wittmann-Liebold B. FEBS Lett. 245:253-260(1989). [2] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

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548. Ribosomal protein S19 signature

Ribosomal protein S19 is one of the proteins from the small ribosomal subunit. In *Escherichia coli*, S19 is known to form a complex with S13 that binds strongly to 16S ribosomal RNA. S19 belongs to a family of ribosomal proteins

- 15 which, on the basis of sequence similarities [1,2], groups: - Eubacterial S19.
 - Algal and plant chloroplast S19. - Cyanelle S19. - Archaeobacterial S19.
 - Plant mitochondrial S19. - Eukaryotic S15 ('rig' protein).

S19 is a protein of 88 to 144 amino-acid residues. Our signature pattern is based on the few conserved positions located in the C-terminal section of these proteins.

- 20 -Consensus pattern: [STDNQ]-G-[KROM]-x(6)-[LIVM]-x(4)-[LIVM]-[GSD]-x(2)-[LF]-[GAS]-[DE]-F-x(2)-[ST]
 [1] Kitagawa M., Takasawa S., Kikuchi N., Itoh T., Teraoka H., Yamamoto H., Okamoto H. FEBS Lett. 283:210-214(1991).
 25 [2] Otaka E., Hashimoto T., Mizuta K.
 Protein Seq. Data Anal. 5:285-300(1993).

549. Ribosomal protein S19e signature

- 30 A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities [1,2]. One of these families consists of: - Mammalian S19. - *Drosophila* S19.
 - *Ascaris lumbricoides* S19g (ALEP-1) and S19s. - Yeast YS16 (RP55A and RP55B).
 - *Aspergillus* S16. - *Halobacterium marismortui* HS12.

These proteins have 143 to 155 amino acids.

A well conserved stretch of 20 residues in the C-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: P-x(6)-[SAN]-x(2)-[LIVMA]-x-R-x-[ALIV]-[LV]-Q-x-L-[EQ]

- 5 [1] Etter A., Aboutanos M., Tobler H., Mueller F.

Proc. Natl. Acad. Sci. U.S.A. 88:1593-1596(1991).

[2] Suzuki K., Olvera J., Wool I.G. Biochimie 72:299-302(1990).

10 550. Ribosomal protein S2 signatures

Ribosomal protein S2 is one of the proteins from the small ribosomal subunit.

S2 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S2. - Algal and plant chloroplast S2.

- Cyanelle S2. - Archaeobacterial S2.

- 15 - Higher eukaryotes P40 (previously thought to be a laminin receptor).

- Yeast NAB1. - Plant mitochondrial S2. - Yeast mitochondrial MRP4.

S2 is a protein of 235 to 394 amino-acid residues.

Two conserved regions have been selected as signature patterns. One is located in the N-terminal section and the other in the central section.

- 20 -Consensus pattern: [LIVMFMA]-x(2)-[LIVMFYC](2)-x-[STAC]-[GSTANQEK]-[STALV]-[HY]-[LIVMF]-G

-Consensus pattern: P-x(2)-[LIVMF](2)-[LIVMS]-x-[GDN]-x(3)-[DENL]-x(3)-[LIVM]-x-E-x(4)-[GNQKRH]-[LIVM]-[AP]

[1] Davis S.C., Tzagoloff A., Ellis S.R.

- 25 J. Biol. Chem. 267:5508-5514(1992).

[2] Tohgo A., Takasawa S., Munakata H., Yonekura H., Hayashi N., Okamoto H. FEBS Lett. 340:133-138(1994).

30 551. Ribosomal protein S21 signature

Ribosomal protein S21 is one of the proteins from the small ribosomal subunit. So far S21 has only been found in eubacteria. It is a protein of 55 to 70 amino-acid residues. A conserved region in the N-terminal section of the protein has been selected as a signature pattern.

Consensus pattern: [DE]-x-A-[LIY]-[KR]-R-F-K-[KR]-x(3)-[KR]

552. Ribosomal protein S21e signature

- 5 A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of: - Mammalian S21 [1].
- *Caenorhabditis elegans* S21 (F37C12.11). - Rice S21 [2].
- Yeast S21 (Ys25) [3]. - Fission yeast S28 [4].

These proteins have 82 to 87 amino acids.

- 10 A perfectly conserved nonapeptide in the N-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: L-Y-V-P-R-K-C-S-[SA]

[1] Bhat K.S., Morrison S.G. Nucleic Acids Res. 21:2939-2939(1993).

[2] Nishi R., Hashimoto H., Uchimiya H., Kato A.

- 15 Biochim. Biophys. Acta 1216:113-114(1993).[3] Suzuki K., Otaka E.
Nucleic Acids Res. 16:6223-6223(1988).[4] Itoh T., Okata E., Matsui K.A.
Biochemistry 24:7418-7423(1985).

- 20 553. Ribosomal protein S24e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrate S24 [1]. - Yeast Rp50. - *Mucor racemosus* S24 [2].
- *Halobacterium marismortui* HS15 [3]. - *Methanococcus jannaschii* MJ0394.

- 25 These proteins have 101 to 148 amino acids.

A well conserved stretch in the central part of these proteins has been selected as a signature pattern.

-Consensus pattern: [FYA]-G-x(2)-[KR]-[STA]-x-G-[FY]-[GA]-x-[LIVM]-Y-[DN]-[SDN]

- 30 [1] Brown S.J., Jewell A., Maki C.G., Roufa D.J. Gene 91:293-296(1990).

[2] Sosa L., Fonzi W.A., Sypherd P.S.

Nucleic Acids Res. 17:9319-9331(1989).[3] Kimura J., Arndt E., Kimura M.
FEBS Lett. 224:65-70(1987).

554. Ribosomal protein S26e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of: - Mammalian S26 [1].

- 5 - Octopus S26 [2]. - Drosophila S26 (DS31) [3]. - Plant cytoplasmic S26.
- Fungi S26 [4].

These proteins have 114 to 127 amino acids.

A conserved octapeptide in the central part of these proteins has been selected as a signature pattern.

- 10 -Consensus pattern: [YH]-C-V-S-C-A-I-H

[1] Kuwano Y., Nakanishi O., Nabeshima Y., Tanaka T., Ogata K.

J. Biochem. 97:983-992(1985).[2] Zinov'eva R.D., Tomarev S.I.

Dokl. Akad. Nauk SSSR 304:464-469(1989).

[3] Itoh N., Ohta K., Ohta M., Kawasaki T., Yamashina I.

- 15 Nucleic Acids Res. 17:2121-2121(1989).[4] Wu M., Tan H.
Gene 150:401-402(1994).

555. Ribosomal protein S28e signature

- 20 A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian S28 [1]. - Plant S28 [2]. - Fungi S33 [3].

- Methanococcus jannaschii MJ1202.

These proteins have from 64 to 78 amino acids.

- 25 A highly conserved nonapeptide from the C-terminal extremity of these proteins has been selected as a signature pattern.

-Consensus pattern: E-[ST]-E-R-E-A-R-x-L

[1] Chan Y.-L., Olvera J., Wool I.G.

Biochem. Biophys. Res. Commun. 179:314-318(1991).

- 30 [2] Hwang I., Goodman H.M. Plant Physiol. 102:1357-1358(1993).

[3] Hoekstra R., Ferreira P.M., Bootsman T.C., Mager W.H., Planta R.J.

Yeast 8:949-959(1992).

556. Ribosomal protein S3Ae signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian S3A (originally known as v-fos transformation effector protein).
- *Caenorhabditis elegans* S3A (F56F3.5).
- Plant cytoplasmic S3A (CYC07) [1].
- Yeast Rp10 (PLC1 and PLC2).
- Fission yeast Rp10 (SpAC13G6.02c).
- *Methanococcus jannaschii* MJ0980.

These proteins have from 220 to 250 amino acids.

A conserved stretch in their N-terminal section was selected as a signature pattern.

-Consensus pattern: [LIV]-x-[GH]-R-[IV]-x-E-x-[SC]-L-x-D-L

[1] Liu J.H., Reid D.M.

Plant Physiol. 109:338-338(1995).

557. Ribosomal protein S3 signature

Ribosomal protein S3 is one of the proteins from the small ribosomal subunit.

In *Escherichia coli*, S3 is known to be involved in the binding of initiator Met-tRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial S3.

- Algal and plant chloroplast S3. - Cyanelle S3. - Archaeobacterial S3.
- Plant mitochondrial S3. - Vertebrate S3. - Insect S3.
- *Caenorhabditis elegans* S3 (C23G10.3). - Yeast S3 (Rp13).

S3 is a protein of 209 to 559 amino-acid residues.

A conserved region located in the C-terminal section has been selected as a signature pattern.

-Consensus pattern: [GSTA]-[KR]-x(6)-G-x-[LIVMT]-x(2)-[NQSCH]-x(1,3)-[LIVFCA]-x(3)-[LIV]-[DENQ]-x(7)-[LMT]-x(2)-G-x(2)-G

[1] Otake E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

558. Ribosomal protein S4 signature

Ribosomal protein S4 is one of the proteins from the small ribosomal subunit.

In *Escherichia coli*, S4 is known to bind directly to 16S ribosomal RNA.

Mutations in S4 have been shown to increase translational error frequencies.

It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S4. - Algal and plant chloroplast S4.

- Cyanelle S4. - Archaeobacterial S4. - Mammalian S9. - Yeast YS11 (SUP45).
- Marchantia polymorpha mitochondrial S4. - Dictyostelium discoideum rp1024.

5 - Yeast protein NAM9 [3]. NAM9 has been characterized as a suppressor for ochre mutations in mitochondrial DNA. It could be a ribosomal protein that acts as a suppressor by decreasing translation accuracy.

S4 is a protein of 171 to 205 amino-acid residues (except for NAM9 which is much larger). The signature pattern for this protein is based on a conserved
10 region located in the central section of these proteins.

-Consensus pattern: [LIVM]-[DE]-x-R-[LI]-x(3)-[LIVMC]-[VMFYHQ]-[KRT]-x(3)-[STAGCVF]-x-[ST]-x(3)-[SAI]-[KR]-x-[LIVMF](2)

[1] Mizuta K., Hashimoto T., Suzuki K.I., Otake E.

Nucleic Acids Res. 19:2603-2608(1991).

15 [2] Otake E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

[3] Boguta M., Dmochowska A., Borsuk P., Wrobel K., Gargouri A., Lazowska J., Slonimski P., Szczesniak B., Kruszewska A.

Mol. Cell. Biol. 12:402-412(1992).

20 559. Ribosomal protein S4e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

25 - Mammalian S4 [1]. Two highly similar isoforms of this protein exist : one coded by a gene on chromosome Y, and the other on chromosome X.

- Plant cytoplasmic S4 [2] - Yeast S7 (YS6). - Archeobacterial S4e.

These proteins have 233 to 264 amino acids.

A highly conserved stretch of 15 residues in their N-terminal section has
30 been selected as a signature pattern. Four positions in this region are positively charged residues.

-Consensus pattern: H-x-K-R-[LIVMF]-[SANK]-x-P-x(2)-[WY]-x-[LIVM]-x-[KRP]

[1] Fisher E.M., Beer-Romero P., Brown L.G., Ridley A., McNeil J.A.,

Lawrence J.B., Willard H.F., Bieber F.R., Page D.C.

Cell 63:1205-1218(1990).

[2] Braun H.P., Emmermann M., Mentzel H., Schmitz U.K.

Biochim. Biophys. Acta 1218:435-438(1994).

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560. Ribosomal protein S5 signature

Ribosomal protein S5 is one of the proteins from the small ribosomal subunit. In *Escherichia coli*, S5 is known to be important in the assembly and function of the 30S ribosomal subunit. Mutations in S5 have been shown to increase

10

translational error frequencies. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S5.

- Cyanelle S5. - Red algal chloroplast S5. - Archaeobacterial S5.

- Mammalian S2 (LLrep3). - *Caenorhabditis elegans* S2 (C49H3.11).

- *Drosophila* S2. - Plant S2. - Yeast S4 (SUP44). - Fungi mitochondrial S5.

15

S5 is a protein of 166 to 254 amino-acid residues. The signature pattern for this protein is based on a conserved region, rich in glycine residues, and located in the N-terminal section of these proteins.

-Consensus pattern: G-[KRO]-x(3)-[FY]-x-[ACV]-x(2)-[LIVMA]-[LIVM]-[AG]-[DN]-x(2)-G-x-[LIVM]-G-x-[SAG]-x(5,6)-[DEQ]-[LIVMA]-x(2)-A-

20

[LIVMF]

[1] All-Robyn J.A., Brown N., Otaka E., Liebman S.W.

Mol. Cell. Biol. 10:6544-6553(1990), [2] Otaka E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

25

561. Ribosomal protein S6 signature

Ribosomal protein S6 is one of the proteins from the small ribosomal subunit. In *Escherichia coli*, S6 is known to bind together with S18 to 16S ribosomal RNA. It belongs to a family of ribosomal proteins which, on the basis of

30

sequence similarities, groups: - Eubacterial S6. - Red algal chloroplast S6.

- Cyanelle S6.

S6 is a protein of 95 to 208 amino-acid residues. The signature pattern for this protein is based on a conserved region located in the N-terminal section of these proteins.

-Consensus pattern: G-x-[KRC]-[DENQRH]-L-[SA]-Y-x-I-[KRNSA]

562. Ribosomal protein S6e signature

- 5 A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian S6 [1]. - *Drosophila* S6 [2]. - Plant S6 [3]. - Yeast S10 (YS4).
- *Halobacterium marismortui* HS13 [4]. - *Methanococcus jannaschii* MJ1260.

- 10 S6 is the major substrate of protein kinases in eukaryotic ribosomes [5]; it may have an important role in controlling cell growth and proliferation through the selective translation of particular classes of mRNA.

These proteins have 135 to 249 amino acids.

A conserved stretch of 12 residues in the N-terminal part of these proteins has been selected as a signature pattern.

- 15 -Consensus pattern: [LIVM]-[STAMR]-G-G-x-D-x(2)-G-x-P-M

[1] Franco R., Rosenfeld M.G. J. Biol. Chem. 265:4321-4325(1990).

[2] Watson K.L., Konrad K.D., Woods D.F., Bryant P.J.

Proc. Natl. Acad. Sci. U.S.A. 89:11302-11306(1992).

[3] Hansen G., Estruch J.J., Spena A.

- 20 Nucleic Acids Res. 20:5230-5230(1992).

[4] Kimura M., Arndt E., Hatakeyama T., Hatakeyama T., Kimura J.

Can. J. Microbiol. 35:195-199(1989).

[5] Bandi H.R., Ferrari S., Krieg J., Meyer H.E., Thomas G.

J. Biol. Chem. 268:4530-4533(1993).

563. Ribosomal protein S7 signature

Ribosomal protein S7 is one of the proteins from the small ribosomal subunit.

- 30 In *Escherichia coli*, S7 is known to bind directly to part of the 3' end of 16S ribosomal RNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3], groups: - Eubacterial S7.

- Algal and plant chloroplast S7. - Cyanelle S7. - Archaebacterial S7.

- Plant mitochondrial S7. - Mammalian S5. - Plant S5.

- *Caenorhabditis elegans* S5 (T05E11.1).

The best conserved region located in the N-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: [DENSEK]-x-[LIVMDET]-x(3)-[LIVMFTA](2)-x(6)-G-K-[KR]-x(5)-[LIVMF]-[LIVMFC]-x(2)-[STAC]

- 5 [1] Klusmann S., Franke P., Bergmann U., Kostka S., Wittmann-Liebold B.
Biol. Chem. Hoppe-Seyler 374:305-312(1993).
 - [2] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).
 - [3] Ignatovich O., Cooper M., Kulesza H.M., Beggs J.D.
- 10 Nucleic Acids Res. 23:4616-4619(1995).

564. Ribosomal protein S7e signature

- A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities [1]. One of these families consists of:
- 15
 - Mammalian S7.
 - Xenopus S8.
 - Insect S7.
 - Yeast probable ribosomal protein S7 (N2212).
 - 20
 - Fission yeast probable ribosomal protein S7 (SpAC18G6.13c).

These proteins have about 200 amino acids. A highly conserved stretch of 14 residues which is located in the central section and which is rich in charged residues was selected as a signature pattern.

- 25 Consensus pattern: [KR]-L-x-R-E-L-E-K-K-F-[SAP]-x-[KR]-H

[1] Salazar C.E., Mills-Hamm D.M., Kumar V., Collins F.H. Nucleic Acids Res. 21:4147-4147(1993).

30

565. Ribosomal protein S8 signature

Ribosomal protein S8 is one of the proteins from the small ribosomal subunit. In *Escherichia coli*, S8 is known to bind directly to 16S ribosomal RNA. It belongs to a family of ribosomal proteins which, on the basis of sequence

similarities [1], groups: - Eubacterial S8. - Algal and plant chloroplast S8.

- Cyanelle S8. - Archaeobacterial S8. - Marchantia polymorpha mitochondrial S8.

- Mammalian S15A. - Plant S15A. - Yeast S22 (S24).

The best conserved region located in the C-terminal section of these proteins

5 has been selected as a signature pattern.

-Consensus pattern: [GE]-x(2)-[LIV](2)-[STY]-[ST]-x(2)-G-[LIVM](2)-x(4)-[AG]-
[KRHAYI]

[1] Otaka E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

10

566. Ribosomal protein S8e signature

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities [1]. One of these families consists of:

15 - Mammalian S8. - *Caenorhabditis elegans* S8 (F42C5.8). - *Leishmania major* S8.

- Plant S8. - Yeast S8 (S14) (Rp19). - Archeobacterial S8e.

These proteins have either about 220 amino acids (in eukaryotes) or about 125 amino acids (in archeobacteria). A conserved stretch which is located in the N-terminal section and which is rich in positively charged residues has

20 been selected as a signature pattern.

-Consensus pattern: [KR]-x(2)-[ST]-G-[GA]-x(5)-[HR]-[KG]-[KR]-x-K-x-E-[LM]-G

[1] Engemann S., Herfurth E., Briesemeister U., Wittmann-Liebold B.

J. Protein Chem. 14:189-195(1995).

25

567. Ribosomal protein S9 signature

Ribosomal protein S9 is one of the proteins from the small ribosomal subunit.

It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S9. - Algal chloroplast S9.

30 - Cyanelle S9. - Archaeobacterial S9. - Mammalian S16. - Plant S16.

- Yeast mitochondrial ribosomal S9.

A conserved region containing many charged residues and located in the central section of these proteins has been selected as a signature pattern.

-Consensus pattern: G-G-G-x(2)-[GSA]-Q-x(2)-[SA]-x(3)-[GSA]-x-[GSTAV]-[KR]-

[GSAL]-[LIF]

[1] Chan Y.-L., Paz V., Olvera J., Wool I.G. FEBS Lett. 263:85-88(1990).

[2] Otake E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

5

568. Ribulose-phosphate 3-epimerase family signatures

Ribulose-phosphate 3-epimerase (EC 5.1.3.1) (also known as pentose-5-phosphate 3-epimerase or PPE) is the enzyme that converts D-ribulose 5-phosphate into

- 10 D-xylulose 5-phosphate in Calvin's reductive pentose phosphate cycle. In *Alcaligenes eutrophus* two copies of the gene coding for PPE are known [1], one is chromosomally encoded (cbbEC), the other one is on a plasmid (cbbEP). PPE has been found in a wide range of bacteria, archaebacteria, fungi and plants. The sequence of PPE is highly related to:

- 15 - *Escherichia coli* D-allulose-6-phosphate 3-epimerase (gene *alsE*).
 - *Escherichia coli* protein *sgcE*.
 - *Mycoplasma genitalium* hypothetical protein MG112.

All these proteins have from 209 to 241 amino acid residues.

- 20 Two conserved regions which are located respectively in the N-terminal and in the central part of these proteins have been selected as signature patterns.

-Consensus pattern: [LIVMF]-H-[LIVMFY]-D-[LIVM]-x-D-x(1,2)-[FY]-[LIVM]-x-N-x-[STAV]

-Consensus pattern: [LIVMA]-x-[LIVM]-M-[ST]-[VS]-x-P-x(3)-G-Q-x-F-x(6)-[NK]-[LIVMC]

- 25 [1] Kusian B., Yoo J.G., Bednarski R., Bowien B.
 J. Bacteriol. 174:7337-7344(1992).

569. (Ricin B lectin) Similarity to lectin domain of ricin beta-chain, 3 copies.

30

This family consists of a triplicated domain involved in cell agglutination in ricin.

570. (Rotamase) PpiC-type peptidyl-prolyl cis-trans isomerase signature Peptidyl-prolyl cis-trans isomerase (EC 5.2.1.8) (PPIase or rotamase) is an enzyme that accelerates protein folding by catalyzing the cis-trans isomerization of proline imidic peptide bonds in oligopeptides [1]. Most characterized PPIases belong to two families, the cyclophilin-type (see <PDOC00154>) and the FKBP-type (see <PDOC00426>). Recently a third family has been discovered [2,3]. So far, the only biochemically characterized member of this family is the *Escherichia coli* protein parvulin (gene *ppiC*), a small (92 residues) cytoplasmic enzyme that prefers amino acid residues with hydrophobic side chains like leucine and phenylalanine in the P1 position of the peptides substrates. PpiC is evolutionary related to a number of proteins that are also probably PPIases:

- *Escherichia coli* and *Haemophilus influenzae* *ppiD*. PpiD is a PPIase which contains a periplasmic *ppiC*-like domain anchored to the inner membrane and which seems to be involved in the folding of outer membrane proteins.

- *Escherichia coli* *surA*. *SurA* is a periplasmic protein that contains two *ppiC*-like domains.

- Nitrogen-assimilating bacteria protein *nifM* which is involved in the activation and stabilization of the iron-component (*nifH*) of nitrogenase.

- *Bacillus subtilis* protein *prsA*, a membrane-bound lipoprotein involved in protein export.

- *Lactococcus* and *Lactobacillus* protease maturation protein *prtM*, a membrane-bound lipoprotein involved in the maturation of a secreted serine proteinase. - Yeast protein *ESS1/PTF1* (processing/termination factor 1).

- *Drosophila* protein *dodo* (gene *dod*). - Mammalian protein *PIN1*,

- *Campylobacter jejuni* cell binding factor 2 (*CBF2*), a secreted antigen.

- *Bacillus subtilis* hypothetical protein *yacD*.

- *Helicobacter pylori* hypothetical protein *HP0175*.

- A hypothetical slime mold protein.

A conserved region that contains a serine which could play a role in the catalytic mechanism of these enzymes has been selected as a signature pattern.

-Consensus pattern: F-[GSADEI]-x-[LVAQ]-A-x(3)-[ST]-x(3,4)-[STQ]-x(3,5)-[GER]-G-x-[LIVM]-[GS]

[1] Fischer G., Schmid F.X.

Biochemistry 29:2205-2212(1990).

[2] Rudd K.E., Sofia H.J., Koonin E.V., Plunkett G. III, Lazar S.,
Rouviere P.E. Trends Biochem. Sci. 20:14-15(1995).

[3] Rahfeld J.-U., Ruecknagel K.P., Schelbert B., Ludwig B., Hacker J.,

5 Mann K., Fischer G. FEBS Lett. 352:180-184(1994).

571. (RnaAD) Ribosomal RNA adenine dimethylases signature

A number of enzymes responsible for the dimethylation of adenosines if
10 ribosomal RNAs (EC 2.1.1.48) have been found [1,2] to be evolutionary related.

These enzymes are:

- Bacterial 16S rRNA dimethylase (gene ksgA), which acts in the biogenesis
of ribosomes by catalyzing the dimethylation of two adjacent adenosines in
the loop of a conserved hairpin near the 3'-end of 16S rRNA. Inactivation
15 of ksgA leads to resistance to the aminoglycoside antibiotic kasugamycin.
- Yeast 18S rRNA dimethylase (gene DIM1), which is functionally similar to
ksgA and that dimethylates twin adenosines in the 3'-end of 18S rRNA.
- Bacterial 'erm' methylases. These enzymes confer resistance to macrolide-
lincosamide-streptogramin B (MLS) antibiotics - such as erythromycin - by
20 dimethylating the adenine residue at position 2058 of 23S rRNA thus
resulting in a reduced affinity between ribosomes and the MLS antibiotics.
- *Caenorhabditis elegans* hypothetical protein EO2H1.1.

The best conserved regions in these enzymes is located in the N-terminal
section and corresponds to a region that is probably involved in S-adenosyl
25 methionine (SAM) binding.

-Consensus pattern: [LIVM]-[LIVMFY]-[DE]-x-G-[STAPV]-G-x-[GA]-x-[LIVMF]-[ST]-
x(2)-[LIVM]-x(6)-[LIVMY]-x-[STAGV]-[LIVMFYHC]-E-x-D

[1] van Gemen B., van Knippenberg P.H.

(In) Nucleic acid methylation, Clawson G.A., Willis D.B., Weissbach A.,
30 Jones P.A., Eds., pp.19-36, Alan R. Liss Inc, New-York, (1990).

[2] Lafontaine D., Delcour J., Glasser A.L., Desgres J., Vandenhoute J.
J. Mol. Biol. 241:492-497(1994).

572. (RuBisCO small) Ribulose biphosphate carboxylase, small chain. 206 members

573. ATP/GTP-binding site motif A (P-loop) (ras)

- 5 From sequence comparisons and crystallographic data analysis it has been shown [1,2,3,4,5,6] that an appreciable proportion of proteins that bind ATP or GTP share a number of more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region, which typically forms a flexible loop between a beta-strand and an alpha-helix. This loop interacts with one of the phosphate groups of the nucleotide. This sequence motif is generally referred to as the 'A' consensus sequence [1] or the 'P-loop' [5]. There are numerous ATP- or GTP-binding proteins in which the P-loop is found. A number of protein families for which the relevance of the presence of such a motif has been noted are listed below: - ATP synthase alpha and beta subunits. - Myosin heavy chains. - Kinesin heavy chains and kinesin-like proteins. - Dynamins and dynamin-like proteins - Guanylate kinase - Thymidine kinase (-
- 10 Thymidylate kinase. - Shikimate kinase. - Nitrogenase iron protein family (nifH/frxC) - ATP-binding proteins involved in 'active transport' (ABC transporters) [7] - DNA and RNA helicases [8,9,10]. - GTP-binding elongation factors (EF-Tu, EF-1alpha, EF-G, EF-2, etc.). - Ras family of GTP-binding proteins (Ras, Rho, Rab, Ral, Ypt1, SEC4, etc.). - Nuclear protein ran. - ADP-ribosylation factors family - Bacterial dnaA protein - Bacterial recA protein -
- 15 Bacterial recF protein - Guanine nucleotide-binding proteins alpha subunits (Gi, Gs, Gt, G0, etc.). - DNA mismatch repair proteins mutS family - Bacterial type II secretion system protein E. Not all ATP- or GTP-binding proteins are picked-up by this motif. A number of proteins escape detection because the structure of their ATP-binding site is completely different from that of the P-loop. Examples of such proteins are the E1-E2 ATPases or the
- 20 glycolytic kinases. In other ATP- or GTP-binding proteins the flexible loop exists in a slightly different form; this is the case for tubulins or protein kinases. A special mention must be reserved for adenylate kinase, in which there is a single deviation from the P-loop pattern: in the last position Gly is found instead of Ser or Thr.
- Consensus pattern: [AG]-x(4)-G-K-[ST]
- 30 In addition to the proteins listed above, the 'A' motif is also found in a number of other proteins. Most of these proteins probably bind a nucleotide, but others are definitively not ATP- or GTP-binding (as for example chymotrypsin, or human ferritin light chain).
- [1] Walker J.E., Saraste M., Runswick M.J., Gay N.J. EMBO J. 1:945-951(1982). [2] Moller W., Amons R. FEBS Lett. 186:1-7(1985). [3] Fry D.C., Kuby S.A., Mildvan A.S. Proc. Natl.

- Acad. Sci. U.S.A. 83:907-911(1986).[4] Dever T.E., Glynnias M.J., Merrick W.C. Proc. Natl. Acad. Sci. U.S.A. 84:1814-1818(1987).[5] Saraste M., Sibbald P.R., Wittinghofer A. Trends Biochem. Sci. 15:430-434(1990).[6] Koonin E.V. J. Mol. Biol. 229:1165-1174(1993).[7] Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P. J. Bioenerg.
- 5 Biomembr. 22:571-592(1990).[8] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).[9] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).[10] Gorbalenya A.E., Koonin E.V., Donchenko A.P., Blinov V.M. Nucleic Acids Res. 17:4713-4730(1989).
- 10 GTP-binding nuclear protein ran signature (ras)
- Ran (or TC4) is a small abundant nuclear protein that binds and hydrolyzes GTP and which has been implicated in a large number of processes including nucleocytoplasmic transport, RNA synthesis, processing and export and cell cycle checkpoint control [1,2]. Ran is generally included in the RAS 'superfamily' of small GTP-binding proteins [3], but it is only
- 15 slightly related to the other RAS proteins. It also differs from RAS proteins in that it lacks cysteine residues at its C- terminal and is therefore not subject to prenylation. Instead ran has an acidic C-terminus. It is, however similar to RAS family members in requiring a specific guanine nucleotide exchange factor (GEF) and a specific GTPase activating protein (GAP) as stimulators of overall GTPase activity. The region of the GTP-binding B motif which, in ran,
- 20 is perfectly conserved has been selected as a signature pattern.
- Consensus pattern: D-T-A-G-Q-E-K-[LF]-G-G-L-R-[DE]-G-Y-Y- Proteins belonging to this family also contain a copy of the ATP/GTP- binding motif 'A' (P-loop).
- [1] Scheffzek K., Klebe C., Fritz-Wolf K., Kabsch W., Wittinghofer A. Nature 374:378-381(1995).[2] Rush M.G., Drivas G., d'Eustachio P. BioEssays 18:103-112(1996).[3]
- 25 Valencia A., Chardin P., Wittinghofer A., Sander C. Biochemistry 30:4637-4648(1991).

574. recA signature

- The bacterial recA protein [1,2,3,E1] is essential for homologous recombination and
- 30 recombinational repair of DNA damage. RecA has many activities: it filaments, it binds to single- and double-stranded DNA, it binds and hydrolyzes ATP, it is also a recombinase and, finally, it interacts with lexA causing its activation and leading to its autocatalytic cleavage. RecA is a protein of about 350 amino-acid residues. Its sequence is very well conserved [3,4,5,E1] among eubacterial species. It is also found in the chloroplast of plants [6]. The best

conserved region, a nonapeptide located in the middle of the sequence which is part of the monomer-monomer interface in a recA filament has been selected as a signature pattern.

Consensus pattern: A-L-[KR]-[IF]-[FY]-[STA]-[STAD]-[LIVMQ]-R-

[1] Smith K.C., Wang T.-C. V. BioEssays 10:12-16(1989).[2] Lloyd A.T., Sharp P.M. J.

- 5 Mol. Evol. 37:399-407(1993).[3] Roca A.I., Cox M.M. Prog. Nucleic Acids Res. Mol. Biol. 56:129-223(1997).[4] Karlin S., Weinstock G.M., Brendel V. J. Bacteriol. 177:6881-6893(1995).[5] Eisen J.A. J. Mol. Evol. 41:1105-1123(1995).[6] Cerutti H.D., Osman M., Grandoni P., Jagendorf A.T. Proc. Natl. Acad. Sci. U.S.A. 89:8068-8072(1992).[E1] <http://www.tigr.org/~jeisen/RecA/RecA.html>

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575. Response regulator receiver domain

This domain receives the signal from the sensor partner inComment: bacterial two-component systems. It is usually found N-terminalComment: to a DNA binding effector domain.

15

[1] Pao GM, Saier MH; J Mol Evol 1995;40:136-154.

576. Ribonucleotide reductase large subunit signature

- 20 *Ribonucleotide reductase (EC 1.1.7.4.1) [1,2] catalyzes the reductive synthesis of deoxyribonucleotides from their corresponding ribonucleotides. It provides the precursors necessary for DNA synthesis. Ribonucleotide reductase is an oligomeric enzyme composed of a large subunit (700 to 1000 residues) and a small subunit (300 to 400 residues). There are regions of similarities in the sequence of the large chain from prokaryotes, eukaryotes and
- 25 viruses. One of these regions has been selected as a signature pattern.

Consensus pattern: W-x(2)-[LF]-x(6,7)-G-[LIVM]-[FYRA]-[NH]-x(3)-[STAQLIVM]-[ASC]-x(2)-[PA]-

[1] Nillson O., Lundqvist T., Hahne S., Sjoberg B.-M. Biochem. Soc. Trans. 16:91-94(1988).[2] Reichard P. Science 260:1773-1777(1993).

30

577. Ribonuclease T2 family histidine active sites

The fungal ribonucleases T2 from *Aspergillus oryzae*, M from *Aspergillus saitoi* and Rh from *Rhizopus niveus* are structurally and functionally related 30 Kd glycoproteins [1] that cleave

the 3'-5' internucleotide linkage of RNA via a nucleotide 2',3'-cyclic phosphate intermediates (EC 3.1.27.1). A number of other RNases have been found to be evolutionary related to these fungal enzymes: - Self-incompatibility [2] in flowering plants is often controlled by a single gene (S-gene) that has several alleles. This gene prevents fertilization by self-pollen or by pollen bearing either of the two S- alleles expressed in the style. The self-incompatibility glycoprotein from several higher plants of the solanaceae family has been shown [2,3] to be a ribonuclease. - Phosphate-starvation induced RNases LE and LX from tomato [4]. These two enzymes are probably involved in a phosphate-starvation rescue system. - *Escherichia coli* periplasmic RNase I (EC 3.1.27.6) (gene *rna*) [5]. - *Aeromonas hydrophila* periplasmic RNase. - *Haemophilus influenzae* hypothetical protein HI0526. Two histidines residues have been shown [6,7] to be involved in the catalytic mechanism of RNase T2 and Rh. These residues and the region around them are highly conserved in all the sequence described above. Two signature patterns have been developed, one for each of the two active-site histidines. The second pattern also contains a cysteine which is known to be involved in a disulfide bond.

Consensus pattern: [FYWL]-x-[LIVM]-H-G-L-W-P [H is an active site residue]
 Consensus pattern: [LIVMF]-x(2)-[HDGTY]-[EQ]-[FYW]-x-[KR]-H-G-x-C [H is an active site residue] [C is involved in a disulfide bond]

[1] Watanabe H., Naitoh A., Suyama Y., Inokuchi N., Shimada H., Koyama T., Ohgi K., Irie M. J. Biochem. 108:303-310(1990). [2] Haring V., Gray J.E., McClure B.A., Anderson M.A., Clarke A.E. Science 250:937-941(1990). [3] McClure B.A., Haring V., Ebert P.R., Anderson M.A., Simpson R.J., Sakiyama F., Clarke A.E. Nature 342:95957(1989). [4] Loeffler A., Glund K., Irie M. Eur. J. Biochem. 214:627-633(1993). [5] Meador J. III, Kennell D. Gene 95:1-7(1990). [6] Kawata Y., Sakiyama F., Hayashi F., Kyogoku Y. Eur. J. Biochem. 187:255-262(1990). [7] Kurihara H., Mitsui Y., Ohgi K., Irie M., Mizuno H., Nakamura K.T. FEBS Lett. 306:189-192(1992).

578. Ribonucleotide reductase large subunit signature. Ribonucleotide reductase (EC 1.1.7.4) [1,2] catalyzes the reductive synthesis of deoxyribonucleotides from their corresponding ribonucleotides. It provides the precursors necessary for DNA synthesis. Ribonucleotide reductase is an oligomeric enzyme composed of a large subunit (700 to 1000 residues) and a small subunit (300 to 400 residues). There are regions of similarities in the

sequence of the large chain from prokaryotes, eukaryotes and viruses. One of these regions has been developed as a signature pattern.

Consensus pattern: W-x(2)-[LF]-x(6,7)-G-[LIVM]-[FYRA]-[NH]-x(3)-[STAQLIVM]-

5 [ASC]-x(2)-[PA]-

[1] Nilsson O., Lundqvist T., Hahne S., Sjöberg B.-M. Biochem. Soc. Trans. 16:91-94(1988). [2] Reichard P. Science 260:1773-1777(1993).

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579. RNase H

RNase H digests the RNA strand of an RNA/DNA hybrid. Important enzyme in retroviral replication cycle, and often found as a domain associated with reverse transcriptases.

Structure is a mixed alpha+beta fold with three a/b/a layers.

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580. Eukaryotic putative RNA-binding region RNP-1 signature (rrm)

Many eukaryotic proteins that are known or supposed to bind single-stranded RNA contain one or more copies of a putative RNA-binding domain of about 90 amino acids [1,2]. This region has been found in the following proteins: ** Heterogeneous nuclear

20

ribonucleoproteins ** - hnRNP A1 (helix destabilizing protein) (twice). - hnRNP A2/B1 (twice). - hnRNP C (C1/C2) (once). - hnRNP E (UP2) (at least once). - hnRNP G (once). ** Small nuclear ribonucleoproteins ** - U1 snRNP 70 Kd (once). - U1 snRNP A (once). - U2 snRNP B" (once). ** Pre-RNA and mRNA associated proteins ** - Protein synthesis

25

initiation factor 4B (eIF-4B) [3], a protein essential for the binding of mRNA to ribosomes (once). - Nucleolin (4 times). - Yeast single-stranded nucleic acid-binding protein (gene SSB1) (once). - Yeast protein NSR1 (twice). NSR1 is involved in pre-rRNA processing; it specifically binds nuclear localization sequences. - Poly(A) binding protein (PABP) (4 times). ** Others ** - Drosophila sex determination protein Sex-lethal (Sxl) (twice). -

30

Drosophila sex determination protein Transformer-2 (Tra-2) (once). - Drosophila 'elav' protein (3 times), which is probably involved in the RNA metabolism of neurons. - Human paraneoplastic encephalomyelitis antigen HuD (3 times) [4], which is highly similar to elav and which may play a role in neuron-specific RNA processing. - Drosophila 'bicoid' protein (once) [5], a segment-polarity homeobox protein that may also bind to specific mRNAs. - La

antigen (once), a protein which may play a role in the transcription of RNA polymerase III. - The 60 Kd Ro protein (once), a putative RNP complex protein. - A maize protein induced by abscisic acid in response to water stress, which seems to be a RNA-binding protein. - Three tobacco proteins, located in the chloroplast [6], which may be involved in splicing and/or processing of chloroplast RNAs (twice). - X16 [7], a mammalian protein which may be involved in RNA processing in relation with cellular proliferation and/or maturation. - Insulin-induced growth response protein Cl-4 from rat (twice). - Nucleolysins TIA-1 and TIAR (3 times) [8] which possesses nucleolytic activity against cytotoxic lymphocyte target cells. may be involved in apoptosis. - Yeast RNA15 protein, which plays a role in mRNA stability and/or poly-(A) tail length [9]. Inside the putative RNA-binding domain there are two regions which are highly conserved. The first one is a hydrophobic segment of six residues (which is called the RNP-2 motif), the second one is an octapeptide motif (which is called RNP-1 or RNP-CS). The position of both motifs in the domain is shown in the following schematic representation:

xxxxxxx#####xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx#####xxxxxxxxxxxxxxxxxxxxxxxxxxxxx
RNP-2 RNP-1

The RNP-1 motif has been used as a signature pattern for this type of domain.

Consensus pattern: [RK]-G-{EDRKHPG}-[AGSCI]-[FY]-[LIVA]-x-[FYLM] In most cases the residue in position 3 of the pattern is either Tyr or Phe.

[1] Bandziulis R.J., Swanson M.S., Dreyfuss G. Genes Dev. 3:431-437(1989).[2] Dreyfuss G., Swanson M.S., Pinol-Roma S. Trends Biochem. Sci. 13:86-91(1988).[3] Milburn S.C., Hershey J.W.B., Davies M.V., Kelleher K., Kaufman R.J. EMBO J. 9:2783-2790(1990).[4] Szabo A., Dalmau J., Manley G., Rosenfeld M., Wong E., Henson J., Posner J.B., Furneaux H.M. Cell 67:325-333(1991).[5] Rebagliati M. Cell 58:231-232(1989).[6] Li Y., Sugiura M. EMBO J. 9:3059-3066(1990).[7] Ayane M., Preuss U., Koehler G., Nielsen P.J. Nucleic Acids Res. 19:1273-1278(1991).[8] Kawakami A., Tian Q., Duan X., Streuli M., Schlossman S.F., Anderson P. Proc. Natl. Acad. Sci. U.S.A. 89:8681-8685(1992).[9] Minvielle-Sebastia L., Winsor B., Bonneaud N., Lacroute F. Mol. Cell. Biol. 11:3075-3087(1991).

581. Rubredoxin signature

Rubredoxins [1] are small electron-transfer prokaryotic proteins. They contain an iron atom which is ligated by four cysteine residues. Rubredoxins are, in some cases, functionally interchangeable with ferredoxins.

- 5 A conserved region that includes two of the cysteine residues that bind the iron atom has been selected as a pattern for these proteins.

Consensus pattern: [LIVM]-x(3)-W-x-C-P-x-C-[AGD] [The two C's bind the iron atom]

- 10 In *Pseudomonas oleovorans* rubredoxin 2 (gene *alkG*) [2], this pattern is found twice because *alkG* has two rubredoxin domains.

Rubrerhythrin [3], a protein with inorganic pyrophosphatase activity from *Desulfovibrio vulgaris* possesses a C-terminal rubredoxin-like domain, but this domain is too divergent to be detected by the above pattern.

- [1] Berg J.M., Holm R.H.(In) Iron-sulfur proteins, Spiro T.G., Ed., pp1-66, Wiley, New-York, (1982). [2] Kok M., Oldenhuis R., der Linden M.P.G., Meulenberg C.H.C., Kingma J., Witholt B., J. Biol. Chem. 264:5442-5451(1989). [3] van Beeumen J.J., van Driessche G., Liu M.-Y., Le Gall J., J. Biol. Chem. 266:20645-20653(1991).

- 20 582. (rvp) Eukaryotic and viral aspartyl proteases active site
Aspartyl proteases, also known as acid proteases, (EC 3.4.23.-) are a widely distributed family of proteolytic enzymes [1,2,3] known to exist invertebrates, fungi, plants, retroviruses and some plant viruses. Aspartate proteases of eukaryotes are monomeric enzymes which consist of two domains. Each domain contains an active site centered on a catalytic aspartyl residue. The two domains most probably evolved from the duplication of an ancestral gene encoding a primordial domain. Currently known eukaryotic aspartyl proteases are: - Vertebrate gastric pepsins A and C (also known as gastricsin). - Vertebrate chymosin (rennin), involved in digestion and used for making cheese. - Vertebrate lysosomal cathepsins D (EC 3.4.23.5) and E (EC 3.4.23.34). - Mammalian renin (EC 3.4.23.15) whose function is to generate angiotensin I from angiotensinogen in the plasma. - Fungal proteases such as aspergillopepsin A (EC 3.4.23.18), candidapepsin (EC 3.4.23.24), mucoropepsin (EC 3.4.23.23) (mucor rennin), endothiapepsin (EC 3.4.23.22), polyporopepsin (EC 3.4.23.29), and rhizopuspepsin (EC 3.4.23.21). - Yeast saccharopepsin (EC 3.4.23.25) (proteinase A) (gene PEP4). PEP4 is implicated in posttranslational regulation of vacuolar hydrolases. -

Yeast barrier pepsin (EC 3.4.23.35) (gene BAR1); a protease that cleaves alpha-factor and thus acts as an antagonist of the mating pheromone. - Fission yeast *ssa1* which is involved in degrading or processing the mating pheromones. Most retroviruses and some plant viruses, such as badnaviruses, encode for an aspartyl protease which is an homodimer of a chain of about 95 to 125 amino acids. In most retroviruses, the protease is encoded as a segment of a polyprotein which is cleaved during the maturation process of the virus. It is generally part of the pol polyprotein and, more rarely, of the gagpolyprotein. Conservation of the sequence around the two aspartates of eukaryotic aspartyl proteases and around the single active site of the viral proteases allows us to develop a single signature pattern for both groups of protease.

Consensus pattern: [LIVMFGAC]-[LIVMTADN]-[LIVFSA]-D-[ST]-G-[STAV]-[STAPDENQ]-x-[LIVMFSTNC]-x-[LIVMFGTA] [D is the active site residue] -
[1] Foltmann B. *Essays Biochem.* 17:52-84(1981).[2] Davies D.R. *Annu. Rev. Biophys. Chem.* 19:189-215(1990).[3] Rao J.K.M., Erickson J.W., Wlodawer A. *Biochemistry* 30:4663-4671(1991).[4] Rawlings N.D., Barrett A.J. *Meth. Enzymol.* 248:105-120(1995).

583. (rvt) Reverse transcriptase (RNA-dependent DNA polymerase)

A reverse transcriptase gene is usually indicative of a mobile element such as a retrotransposon or retrovirus. Reverse transcriptases occur in a variety of mobile elements, including retrotransposons, retroviruses, group II introns, bacterial msDNAs, hepadnaviruses, and caulimoviruses. Number of members: 1233

[1] Medline: 91006031. Origin and evolution of retroelements based upon their reverse transcriptase sequences. Xiong Y, Eickbush TH; *EMBO J* 1990;9:3353-3362.

584. (S-AdoMet synt) S-adenosylmethionine synthetase signatures

S-adenosylmethionine synthetase (EC 2.5.1.6) is the enzyme that catalyzes the formation of S-adenosylmethionine (AdoMet) from methionine and ATP [1]. AdoMet is an important methyl donor for transmethylation and is also the propylamino donor in polyamine biosynthesis. In bacteria there is a single isoform of AdoMet synthetase (gene *metK*), there are two in budding yeast (genes *SAM1* and *SAM2*) and in mammals while in plants there is generally a multigene family. The sequence of AdoMet synthetase is highly conserved throughout isozymes and species. Two signature patterns have been selected for this type of enzyme; the

first is a hexapeptide which seems to be involved in ATP-binding; the second is an almost perfectly conserved glycine-rich nonapeptide.

Consensus pattern: G-A-G-D-Q-G-x(3)-G-[FYH]-Sequences known to belong to this class detected by the pattern:

- 5 Consensus pattern: G-[GA]-G-[ASC]-F-S-x-K-[DE]

[1] Horikawa S., Sasuga J., Shimizu K., Ozasa H., Tsukada K. J. Biol. Chem. 265:13683-13686(1990).

- 10 585. S1 RNA binding domain

The S1 domain occurs in a wide range of RNAComment: associated proteins. It is structurally similarComment: to cold shock protein which binds nucleic acids.Comment: The S1 domain has an OB-fold structure.

[1] Bycroft M, Hubbard TJ, Proctor M, Freund SM, Murzin AG; Cell 1997;88:235-242.

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586. SAICAR synthetase signatures

Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6)

(SAICARsynthetase) catalyzes the seventh step in the de novo purine biosynthetic pathway; the ATP-dependent conversion of 5'-phosphoribosyl-5-aminoimidazole-4-carboxylic acid and aspartic acid to SAICAR [1]. In bacteria (gene purC),fungi (gene ADE1) and plants, SAICAR synthetase is a monofunctional protein;in higher vertebrates it is the N-terminal domain of a bifunctional enzyme that also catalyze phosphoribosylaminoimidazole carboxylase (AIRC) activity. Two conserved regions in the central section of this enzyme

- 25 have been selected as signature patterns for SAICAR synthetase.

Consensus pattern: [LIVMF](2)-P-[LIVM]-E-x-[LIVM]-[LIVMCA]-R-x(3)-[TA]-G-S-

Consensus pattern: [LIVM]-[LIVMA]-D-x-K-[LIVMFY]-E-F-G

[1] Zalkin H., Dixon J.E. Prog. Nucleic Acid Res. Mol. Biol. 42:259-287(1992).

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587. (SCP) Extracellular proteins SCP/Tpx-1/Ag5/PR-1/Sc7 signatures

A variety of extracellular proteins from eukaryotes have been found to be evolutionary related: - Rodent sperm-coating glycoprotein (SCP), also known as acidic epididymal glycoprotein (AEG) . This protein is thought to be involved in sperm maturation [1]. It is a

- protein of about 220 residues and probably contains eight disulfide bonds. - Mammalian testis-specific protein Tpx-1 [2]. Tpx-1 is highly related to SCP's. - Mammalian glioma pathogenesis-related protein (GliPR). - Lizard heliothermine, a toxin that blocks ryanodine receptors. - Venom allergen 5 (Ag5) from vespid wasps and venom allergen 3 (Ag3) from fire ants. These proteins are potent allergens and are the main cause of allergic reactions to stings from insects of the hymenoptera family [3]. Ag5/3 are proteins of about 200 residues and contain four disulfide bonds. - Plant pathogenesis proteins of the PR-1 family [4]. These proteins are synthesized during pathogen infection or other stress-related responses. They are proteins of about 130 to 140 residues and probably contain three disulfide bonds. - Proteins
- 10 Sc7 and Sc14 from the basidiomycete fungus *Schizophyllum commune*. These extracellular proteins are loosely associated with fruit body hyphal walls [5]. Sc7/14 are proteins of about 180 residues and probably contain two disulfide bonds. - *Ancylostoma* secreted protein from dog hookworm. - Yeast hypothetical proteins YJL078c, YJL079c and YKR013w. The exact function of these proteins is not yet known. Two conserved regions located in their C-
- 15 terminal half have been selected as signature patterns. The second signature contains a cysteine which is known to be involved in a disulfide bond in Ag5.
- Consensus pattern: [GDER]-H-[FYWH]-T-Q-[LIVM](2)-W-x(2)-[STN]
 Consensus pattern: [LIVMFYH]-[LIVMFY]-x-C-[NQRHS]-Y-x-[PARH]-x-[GL]-N-[LIVMFYWDN] [C is involved in a disulfide bond]
- 20 [1] Mizuki N., Kasahara M. *Mol. Cell. Endocrinol.* 89:25-32(1992).[2] Kasahara M., Gutknecht J., Brew K., Spurr N., Goodfellow P.N. *Genomics* 5:527-534(1989).[3] Lu G., Villalba M., Coscia M.R., Hoffman D.R., King T.P. *J. Immunol.* 150:2823-2830(1993).[4] Dixon D.C., Cutt J.R., Klessig D.F. *EMBO J.* 10:1317-1324(1991).[5] Schuren F.H.J., Asgeirsdottir S.A., Kothe E.M., Scheer J.M.J., Wessels J.G.H. *J. Gen. Microbiol.* 139:2083-2090(1993).
- 25

588. SET domain

- SET domains appear to be protein-protein interaction domains. It has been
- 30 demonstrated that SET domains mediate interactions with a family of proteins that display similarity with dual-specificity phosphatases (dsPTPases) [2].
- [1] Tripoulas N, LaJeunesse D, Gildea J, Shearn A; *Genetics* 1996;143:913-928. [2] Cui X, De Vivo I, Slany R, Miyamoto A, Firestein R, Cleary, ML; *Nat Genet* 1998;18:331-337.

589. Src homology 3 (SH3) domain profile

The Src homology 3 (SH3) domain is a small protein domain of about 60 amino-acid residues

- 5 first identified as a conserved sequence in the non-catalytic part of several cytoplasmic protein tyrosine kinases (e.g. Src, Abl, Lck) [1]. Since then, it has been found in a great variety of other intracellular or membrane-associated proteins [2,3,4,5]. The SH3 domain has a characteristic fold which consists of five or six beta-strands arranged as two tightly packed anti-parallel beta sheets. The linker regions may contain short helices [6]. The function of the
- 10 SH3 domain is not well understood. The current opinion is that they mediate assembly of specific protein complexes via binding to proline-rich peptides [7]. In general SH3 domains are found as single copies in a given protein, but there is a significant number of protein with two SH3 domains and a few with 3 or 4 copies. So far, SH3 domains have been identified in the following proteins: - Many vertebrate, invertebrate and retroviral cytoplasmic (non-
- 15 receptor) protein tyrosine kinases. In particular in the Src, Abl, Btk, Csk and ZAP70 families of kinases. - Mammalian phosphatidylinositol-specific phospholipase C-gamma-1 and -2. - Mammalian phosphatidyl inositol 3-kinase regulatory p85 subunit. - Mammalian Ras GTPase-activating protein (GAP). - Adaptor proteins mediating binding of guanine nucleotide exchange factors to growth factor receptors: vertebrate GRB2, Caenorhabditis
- 20 elegans sem-5 and Drosophila DRK. All of which have two SH3 domains. - Mammalian Vav oncoprotein, a guanine nucleotide exchange factor of the CDC24 family. - Some guanine-nucleotide releasing factors of the CDC25 family: yeast CDC25, yeast SCD25, fission yeast ste6. - MAGUK proteins. These proteins consist of at least three types of domains: one or more copies of the DHR domain, a SH3 domain and a C-terminal guanylate kinase domain.
- 25 Members of this family are: Drosophila lethal(1) discs large-1 tumor suppressor protein (gene Dlg1), mammalian tight junction protein ZO-1, vertebrate erythrocyte membrane protein p55, Caenorhabditis elegans protein lin-2, rat protein CASK and mammalian synaptic proteins SAP90/PSD-95, CHAPSIN-110/PSD-93, SAP97/DLG1 and SAP102. - Miscellaneous proteins interacting with vertebrate receptor protein tyrosine kinases: mammalian
- 30 cytoplasmic protein Nck (3 copies), oncoprotein Crk (2 copies). - Chicken Src substrate p80/85 protein (cortactin) and the similar human hemopoietic lineage cell specific protein Hs1. - Mammalian dihydropyridine-sensitive L-type calcium channel beta (regulatory) subunit including the related human myasthenic syndrome antigen B (MSYB). - Mammalian neutrophil cytosolic activators of NADPH oxidase: p47 (NCF-1), p67 (NCF-2), and a

potential homolog from *Caenorhabditis elegans* (B0303.7). NCF-1 and -2 have two copies of the SH3 domain, while B0303.7 has four. - Some myosin heavy chains from amoebae, slime molds and yeast (gene MYO3). - Vertebrate and *Drosophila* spectrin and fodrin alpha-chain. - Human amphiphysin. - Yeast actin-binding protein ABP1. - Yeast actin-binding protein

- 5 SLA1 (3 copies). - Yeast protein BEM1 and the fission yeast homolog scd2 (or ral3) (2 copies). - Yeast BEM1-binding proteins BOI2 (BEB1) and BOB1 (BOI1). - Yeast fusion protein FUS1. - Yeast protein RSV167. - Yeast protein SSU81. - Yeast hypothetical proteins YAR014c (1 copy), YFR024c (1 copy), YHL002w (1 copy), YHR016c (1 copy), YJL020C (1 copy), YHR114w (2 copies) and the fission yeast homolog SpAC12C2.05c. -
- 10 *Caenorhabditis elegans* hypothetical proteins F42H10.3. The profile developed to detect SH3 domains is based on a structural alignment consisting of 5 gap-free blocks and 4 linker regions totaling 62 match positions.
[1] Mayer B.J., Hamaguchi M., Hanafusa H. Nature 332:272-275(1988).[2] Musacchio A., Gibson T., Lehto V.P., Saraste M. FEBS Lett. 307:55-61(1992).[3] Pawson T., Schlessinger
- 15 J. Curr. Biol. 3:434-442(1993).[4] Mayer B.J., Baltimore D. Trends Cell Biol. 3:8-13(1993).[5] Pawson T. Nature 373:573-580(1995).[6] Kuriyan J., Cowburn D. Curr. Opin. Struct. Biol. 3:828-837(1993).[7] Morton C.J., Campbell I.D. Curr. Biol. 4:615-617(1994).

- 20 590. Serine hydroxymethyltransferase pyridoxal-phosphate attachment site (SHMT)
Serine hydroxymethyltransferase (EC 2.1.2.1) (SHMT) [1] catalyzes the transfer of the hydroxymethyl group of serine to tetrahydrofolate to form 5,10-methylenetetrahydrofolate and glycine. In vertebrates, it exists in acytoplasmic and a mitochondrial form whereas only one form is found in prokaryotes. Serine hydroxymethyltransferase is a pyridoxal-phosphate
- 25 containing enzyme. The pyridoxal-P group is attached to a lysine residue around which the sequence is highly conserved in all forms of the enzyme.
Consensus pattern: [DEH]-[LIVMFY]-x-[STMV]-[GST]-[ST](2)-H-K-[ST]-[LF]-x-G-[PAC]-[RQ]-[GSA]-[GA] [K is the pyridoxal-P attachment site]
[1] Usha R., Savithri H.S., Rao N.A. Biochim. Biophys. Acta 1204:75-83(1994).

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591. SIS domain

SIS (Sugar ISomerase) domains are found in many phosphosugar isomerases and phosphosugar binding proteins.

[1] Teplyakov A, Obmolova G, Badet-Denisot MA, Badet B, Polikarpov I; Structure 1998;6:1047-1055.

5 592. (SKI) Shikimate kinase signature

Shikimate kinase (EC 2.7.1.71) catalyzes the fifth step in the biosynthesis from chorismate of the aromatic amino acids (the shikimate pathway) in bacteria (gene *aroK* or *aroL*), plants and in fungi (where it is part of a multifunctional enzyme which catalyzes five consecutive steps in this pathway). Shikimate kinase is a small protein of about 200 residues. A conserved region that contains a run of three glycines has been selected as a signature pattern. Consensus pattern: [KR]-x(2)-E-x(3)-[LIVMF]-x(8,12)-[LIVMF](2)-[SA]-x-G(3)-x-[LIVMF]. Proteins belonging to this family also contain a copy of the ATP/GTP-binding motif 'A' (P-loop).

15 593. SNAP-25 family

SNAP-25 (synaptosome-associated protein 25 kDa) proteins are components of SNARE complexes. Members of this family contain a cluster of cysteine residues that can be palmitoylated for membrane attachment [2].

20 [1] Brennwald P, Kearns B, Champion K, Keranen S, Bankaitis V, Novick P; Cell 1994;79:245-258. [2] Risinger C, Blomqvist AG, Lundell I, Lambertsson A, Nassel D, Pieribone VA, Brodin L, Larhammar D; J Biol Chem 1993;268:24408-24414.

25 594. SNF2 and others N-terminal domain

This domain is found in proteins involved in a variety of processes including transcription regulation (e.g., SNF2, STH1, brahma, MOT1), DNA repair (e.g., ERCC6, RAD16, RAD5), DNA recombination (e.g., RAD54), and chromatin unwinding (e.g., ISWI) as well as a variety of other proteins with little functional information (e.g., Iodestar, ETL1).

595. Staphylococcal nuclease homologues (Snase)

Present in all three domains of cellular life. Four copies in the transcriptional coactivator p100. These, however, appear to lack the active site residues of Staphylococcal nuclease. Positions 14 (Asp-21), 34 (Arg-35), 39 (Asp-40), 42 (Glu-43) and Comment: 110 (Arg-87)

- 5 [SNase numbering in parentheses] are thought to be involved in substrate-binding and catalysis.

[1] Ponting CP; Protein Sci 1997;6:459-463. [2] Callebaut I, Mornon JP; Biochem J 1997;321:125-132.

10

596. SPRY domainA

SPRY Domain is named from SPIA and the RYanodine Receptor. Domain of unknown function. Distant homologues are domains in Comment: butyrophilin/marenostrin/pyrin homologues.

- 15 [1] Ponting C, Schultz J, Bork P; Trends Biochem Sci 1997;22:193-194.

597. (SQS PSY) Squalene and phytoene synthases signatures

Two different polyisoprene synthases have been shown [1,2,3] to share a number of regions of sequence similarities: - Squalene synthase (EC 2.5.1.21) (farnesyl-diphosphate farnesyltransferase) (SQS), which catalyzes the conversion of two molecules of farnesyl diphosphate (FPP) into squalene. It is the first committed step in the cholesterol biosynthetic pathway. The reaction carried out by SQS is catalyzed in two separate steps: the first is a head-to-head condensation of the two molecules of FPP to form presqualene diphosphate;

- 25 this intermediate is then rearranged in a NADP-dependent reduction, to form squalene. SQS is found in eukaryotes. In yeast it is encoded by the ERG9 gene, in mammals by the FDFT1 gene. SQS seems to be membrane-bound. - Phytoene synthase (EC 2.5.1.-) (PSY), which catalyzes the conversion of two molecules of geranylgeranyl diphosphate (GGPP) into phytoene. It is the second step in the biosynthesis of carotenoids from isopentenyl
30 diphosphate. The reaction carried out by PSY is catalyzed in two separate steps: the first is a head-to-head condensation of the two molecules of GGPP to form prephytoene diphosphate; this intermediate is then rearranged to form phytoene. PSY is found in all organisms that synthesize carotenoids: plants and photosynthetic bacteria as well as some non-photosynthetic bacteria and fungi. In bacteria PSY is encoded by the gene crtB. In plants PSY

is localized in the chloroplast. As it can be seen from the description above, both SQS and PSY share a number of functional similarities which are also reflected at the level of their primary structure. In particular three well conserved regions are shared by SQS and PSY; they could be involved in substrate binding and/or the catalytic mechanism. Signature patterns have been developed for the second and third conserved regions; they are localized in the central part of these enzymes.

Consensus pattern: Y-[CSAM]-x(2)-[VSG]-A-[GSA]-[LIVAT]-[IV]-G-x(2)-[LMSC]-x(2)-[LIV]

Consensus pattern: [LIVM]-G-x(3)-Q-x(2,3)-N-[IF]-x-R-D-[LIVMFY]-x(2)-[DE]-x(4,7)-R-x-[FY]-x-P-

[1] Summers C., Karst F., Charles A.D. Gene 136:185-192(1993), [2] Robinson G.W., Tsay Y.H., Kienzie B.K., Smith-Monroy C.A., Bishop R.W. Mol. Cell. Biol. 13:2706-2727(1993). [3] Roemer S., Huguency P., Bouvier F., Camara B., Kuntz M. Biochem. Biophys. Res. Commun. 196:1414-1421(1993).

598. SRP54-type proteins GTP-binding domain signature

The signal recognition particle (SRP) is an oligomeric complex that mediates targeting and insertion of the signal sequence of exported proteins into the membrane of the endoplasmic reticulum. SRP consists of a 7S RNA and six protein subunits. One of these subunits, the 54 Kd protein (SRP54), is a GTP-binding protein that interacts with the signal sequence when it emerges from the ribosome. The N-terminal 300 residues of SRP54 include the GTP-binding site (G-domain) and are evolutionary related to similar domains in other proteins which are listed below [1]. - Escherichia coli and Bacillus subtilis ffh protein (P48), a protein which seems to be the prokaryotic counterpart of SRP54. Ffh is associated with a 4.5S RNA in the prokaryotic SRP complex. - Signal recognition particle receptor alpha subunit (docking protein), an integral membrane GTP-binding protein which ensures, in conjunction with SRP, the correct targeting of nascent secretory proteins to the endoplasmic reticulum membrane. The G-domain is located at the C-terminal extremity of the protein. - Bacterial fisY protein, a protein which is believed to play a similar role to that of the docking protein in eukaryotes. The G-domain is located at the C-terminal extremity of the protein. - The pilA protein from Neisseria gonorrhoeae which seems to be the homolog of fisY. - A protein from the archaeobacteria Sulfolobus solfataricus. This protein is also believed to be a docking protein. The G-domain is also at the C-terminus. - Bacterial flagellar biosynthesis protein flhF. The

best conserved regions in those domains are the sequence motifs that are part of the GTP-binding site, but as those regions are not specific to these proteins, they were not used as a signature pattern. Instead, a conserved region located at the C-terminal end of the domain was selected.

- 5 Consensus pattern: P-[LIVM]-x-[FYI]-[LIVMAT]-[GS]-x-[GS]-[EQ]-x(4)-[LIVMF]
[1] Althoff S., Selinger D., Wise J.A. Nucleic Acids Res. 22:1933-1947(1994).

599. (STphosphatase) Serine/threonine specific protein phosphatases signature

- 10 Serine/threonine specific protein phosphatases (EC 3.1.3.16) (PP) [1,2,3] are enzymes that catalyze the removal of a phosphate group attached to a serine or evolutionary related. - Protein phosphatase-1 (PP1) is an enzyme of broad specificity. It is inhibited by two thermostable proteins, inhibitor-1 and -2. In mammals, there are two closely related isoforms of PP-1: PP-1alpha and PP-1beta, produced by alternative splicing of the same gene. In
- 15 *Emericella nidulans*, PP-1 (gene bimG) plays an important role in mitosis control by reversing the action of the nimA kinase. In yeast, PP-1 (gene SIT4) is involved in dephosphorylating the large subunit of RNA polymerase II. - Protein phosphatase-2A (PP2A) is also an enzyme of broad specificity. PP2A is a trimeric enzyme that consist of a core composed of a catalytic subunit associated with a 65 Kd regulatory subunit and a third
- 20 variable subunit. In mammals, there are two closely related isoforms of the catalytic subunit of PP2A: PP2A-alpha and PP2A-beta, encoded by separate genes. - Protein phosphatase-2B (PP2B or calcineurin), a calcium-dependent enzyme whose activity is stimulated by calmodulin. It is composed of two subunits: the catalytic A-subunit and the calcium-binding B-subunit. The specificity of PP2B is restricted. In addition to the above-mentioned enzymes,
- 25 some additional serine/threonine specific protein phosphatases have been characterized and are listed below. - Mammalian phosphatase-X (PP-X), and *Drosophila* phosphatase-V (PP-V) which are closely related but yet distinct from PP2A. - Yeast phosphatase PPH3, which is similar to PP2A, but with different enzymatic properties. - *Drosophila* phosphatase-Y (PP-Y), and yeast phosphatases Z1 and Z2 (genes PPZ1 and PPZ2) which are closely related but yet
- 30 distinct from PP1. - *Drosophila* retinal degeneration protein C (gene rdgC), a calcium-binding phosphatase required to prevent light-induced retinal degeneration. - Phages Lambda and Phi-80 ORF-221 which have been shown to have phosphatase activity and are related to mammalian PP's. The best conserved regions in these proteins is a perfectly conserved pentapeptide that can be used as a signature pattern.

Consensus pattern: [LIVM]-R-G-N-H-E-

[1] Cohen P. *Annu. Rev. Biochem.* 58:453-508(1989).[2] Cohen P., Cohen P.T.W. *J. Biol. Chem.* 264:21435-21438(1989).[3] Cohen P.T.W., Brewis N.D., Hughes V., Mann D.J. *FEBS Lett.* 268:355-359(1990).

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600. Translation initiation factor SUI1 signature

In budding yeast (*Saccharomyces cerevisiae*), SUI1 is a translation initiation factor that functions in concert with eIF-2 and the initiator tRNA-Met in directing the ribosome to the proper start site of translation [1]. SUI1 is a protein of 108 residues. Close homologs of SUI1 have been found [2] in mammals, insects and plants. SUI1 is also evolutionary related to hypothetical proteins from *Escherichia coli* (yciH), *Haemophilus influenzae* (HI1225) and *Methanococcus vannielii*. A conserved region in the C-terminal section has been selected as a signature pattern.

- 15 Consensus pattern: [LIVM]-[EQ]-[LIVM]-Q-G-[DEN]-[KHQ]-[KRV]
[1] Yoon H., Donahue T.F. *Mol. Cell. Biol.* 12:248-260(1992).[2] Fields C.A., Adams M.D. *Biochem. Biophys. Res. Commun.* 198:288-291(1994).

- 20 601. (S T dehydratase) Serine/threonine dehydratases pyridoxal-phosphate attachment site
Serine and threonine dehydratases [1,2] are functionally and structurally related pyridoxal-phosphate dependent enzymes: - L-serine dehydratase (EC 4.2.1.13) and D-serine dehydratase (EC 4.2.1.14) catalyze the dehydration of L-serine (respectively D-serine) into ammonia and pyruvate. - Threonine dehydratase (EC 4.2.1.16) (TDH) catalyzes the
25 dehydration of threonine into alpha-ketobuturate and ammonia. In *Escherichia coli* and other microorganisms, two classes of TDH are known to exist. One is involved in the biosynthesis of isoleucine, the other in hydroxamino acid catabolism. Threonine synthase (EC 4.2.99.2) is also a pyridoxal-phosphate enzyme, it catalyzes the transformation of homoserine-phosphate into threonine. It has been shown [3] that threonine synthase is
30 distantly related to the serine/threonine dehydratases. In all these enzymes, the pyridoxal-phosphate group is attached to a lysine residue. The sequence around this residue is sufficiently conserved to allow the derivation of a pattern specific to serine/threonine dehydratases and threonine synthases.

Consensus pattern: [DESH]-x(4,5)-[STVG]-x-[AS]-[FYI]-K-[DLIFSA]-[RVMF]-[GA]-[LIVMGA] [The K is the pyridoxal-P attachment site]

- [1] Ogawa H., Gomi T., Konishi K., Date T., Naakashima H., Nose K., Matsuda Y., Peraino C., Pitot H.C., Fujioka M. J. Biol. Chem. 264:15818-15823(1989).[2] Datta P., Goss T.J., Omnaas J.R., Patil R.V. Proc. Natl. Acad. Sci. U.S.A. 84:393-397(1987).[3] Parsot C. EMBO J. 5:3013-3019(1986).[4] Grabowski R., Hofmeister A.E.M., Buckel W. Trends Biochem. Sci. 18:297-300(1993).

Cysteine synthase/cystathionine beta-synthase P-phosphate attachment site

- 10 Cysteine synthase (CSase) is the pyridoxal-phosphate dependent enzyme responsible [1] for the formation of cysteine from O-acetyl-serine and hydrogen sulfide with the concomitant release of acetic acid. In bacteria such as *Escherichia coli*, two forms of the enzyme are known (genes *cysK* and *cysM*). In plants there are also two forms, one located in the cytoplasm and the other in chloroplasts. Cystathionine beta-synthase [2] catalyzes the first irreversible step in homocysteine transulfuration; the conjugation of homocysteine and serine forming cystathionine. Like CSase it is a pyridoxal-phosphate dependent enzyme. The two types of enzymes are evolutionarily related. The pyridoxal-phosphate group of CSases has been shown to be attached to a lysine residue which is located in the N-terminal section of these enzymes; the sequence around this residue is highly conserved and can be used as a signature pattern to detect this class of enzymes.

Consensus pattern: K-x-E-x(3)-[PA]-[STAGC]-x-S-[IVAP]-K-x-R-x-[STAG]-x(2)-[LIVM] [The 2nd K is the pyridoxal-P attachment site]

- [1] Saito K., Kurosawa M., Murakoshi I. FEBS Lett. 328:111-114(1993).[2] Swaroop M., Bradley K., Ohura T., Tahara T., Roper M.D., Rosenberg L.E., Kraus J.P. J. Biol. Chem. 267:11455-11461(1992).

602. S locus glycop

S-locus glycoprotein family. In Brassicaceae, self-incompatible plants have a self/non-self

- 30 Comment: recognition system. This is sporophytically controlled by multiple alleles at a single locus (S). S-locus glycoproteins, Comment: as well as S-receptor kinases, are in linkage with the S-alleles [1]. Number of members: 128
- [1] Evolutionary aspects of the S-related genes of the Brassica self-incompatibility system: synonymous and nonsynonymous base substitutions. Hinata K, Watanabe M, Yamakawa S,

Satta Y, Isogai A; Genetics 1995;140:1099-1104. [2] Polymorphism of the S-locus glycoprotein gene (SLG) and the S-locus related gene (SLR1) in *Raphanus sativus* L. and self-incompatible ornamental plants in the Brassicaceae. Sakamoto K, Kusaba M, Nishio T; Mol Gen Genet 1998;258:397-403.

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603. (sdh cyt) Succinate dehydrogenase cytochrome b subunit signatures

Succinate dehydrogenase (SDH) is a membrane-bound complex of two main components: a membrane-extrinsic component composed of an FAD-binding flavoprotein and an iron-sulfur protein, and a hydrophobic component composed of a cytochrome B and a membrane anchor protein. The cytochrome b component is a mono heme transmembrane protein [1,2,3] belonging to a family that groups: - Cytochrome b-556 from bacterial SDH (gene *sdhC*). - Cytochrome b560 from the mammalian mitochondrial SDH complex. - Cytochrome b560 subunit encoded in the mitochondrial genome of some algae and in the plant *Marchantia polymorpha*. - Cytochrome b from yeast mitochondrial SDH complex (gene *SDH3* or *CYB3*). - Protein *cyt-1* from *Caenorhabditis*. These cytochromes are proteins of about 130 residues that comprise three transmembrane regions. There are two conserved histidines which may be involved in binding the heme group. Two signature patterns have been developed that include these histidine residues.

- 20 Consensus pattern: R-P-[LIVMT]-x(3)-[LIVM]-x(6)-[LIVMWPK]-x(4)-S-x(2)-H-R-x-[ST]
[H could be a heme ligand]
Consensus pattern: H-x(3)-[GA]-[LIVMT]-R-[HF]-[LIVMF]-x-[FYWM]-D-x-[GVA] [H could be a heme ligand]
[1] Yu L., Wei Y.-Y., Usui S., Yu C.-A. J. Biol. Chem. 267:24508-24515(1992).[2]
25 Abraham P.R., Mulder A., Van't Riet J., Raue H.A. Mol. Gen. Genet. 242:708-716(1994).[3]
Leblanc C., Boyen C., Richard O., Bonnard G., Grienemberger J.M., Kloareg B. J. Mol. Biol. 250:484-495(1995).

30 604. Sec1 family

[1] The Sec1 family: a novel family of proteins involved in synaptic transmission and general secretion. Halachmi N, Lev Z; J Neurochem 1996;66:889-897.

Number of members: 40

605. Protein secE/sec61-gamma signature

In bacteria, the secE protein plays a role in protein export; it is one of the components - with secY and secA - of the preprotein translocase. In eukaryotes, the evolutionary related protein sec61-gamma plays a role in protein translocation through the endoplasmic reticulum; it is part of a trimeric complex that also consist of sec61-alpha and beta [1]. Both secE and sec61-gamma are small proteins of about 60 to 90 amino acids that contain a single transmembrane region at their C-terminal extremity (Escherichia coli secE is an exception, in that it possess an extra N-terminal segment of 60 residues that contains two additional transmembrane domains). The sequence of secE/sec61-gamma is not extremely well conserved, however it is possible to derive a signature pattern centered on a conserved proline located 10 residues before the beginning of the transmembrane domain.

Consensus pattern: [LIVMFY]-x(2)-[DENQGA]-x(4)-[LIVMFTA]-x-[KRV]-x(2)-[KW]-P-x(3)-[SEQ]-x(7)-[LIVT]-[LIVGA]-[LIVFGAST]

[1] Hartmann E., Sommer T., Prehn S., Goerlich D., Jentsch S., Rapoport T.A. Nature 367:654-657(1994).

606. 11-S plant seed storage proteins signature

Plant seed storage proteins, whose principal function appears to be the major nitrogen source for the developing plant, can be classified, on the basis of their structure, into different families. 11-S are non-glycosylated proteins which form hexameric structures [1,2]. Each of the subunits in the hexamer is itself composed of an acidic and a basic chain derived from a single precursor and linked by a disulfide bond. This structure is shown in the following

representation. +-----+ ||

XXXXXXXXXXCXXXXXXXXXXXXXXXXXXXXNGX CXXXXXXXXXXXXXXXXXXXXX ***** <--
 ----Acidic-subunit-----><----Basic-subunit-----> <-----About 480-to-500-
 residues----->'C': conserved cysteine involved in a disulfide bond.*: position of the
 pattern. Proteins that belong to the 11-S family are: pea and broad bean legumins, rape
 cruciferin, rice glutelins, cotton beta-globulins, soybean glycinins, pumpkin 11-S globulin,
 oat globulin, sunflower helianthinin G3, etc. The region that includes the conserved cleavage
 site between the acidic and basic subunits (Asn-Gly) and a proximal cysteine residue which is
 involved in the interchain disulfide bond have been used as a signature pattern for this family
 of proteins.

Consensus pattern: N-G-x-[DE](2)-x-[LIVMF]-C-[ST]-x(11,12)-[PAG]-D [C is involved in a disulfide bond

[1] Hayashi M., Mori H., Nishimura M., Akazawa T., Hara-Nishimura I. Eur. J. Biochem. 172:627-632(1988).[2] Shorwell M.A., Afonso C., Davies E., Chesnut R.S., Larkins B.A.

5 Plant Physiol. 87:698-704(1988).

607. 7S seed storage protein

7S globulin is one of the main storage proteins of most angiosperms and

10 gymnosperms. The 7S storage proteins are homotrimers.

Number of members: 67

[1] The three-dimensional structure of canavalin from jack bean (Canavalia ensiformis). Ko TP, Ng JD, McPherson A; Plant Physiol 1993;101:729-744.

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608. Aspartate-semialdehyde dehydrogenase signature

Aspartate-semialdehyde dehydrogenase (ASD) catalyzes the second step in the common biosynthetic pathway leading from Asp to diaminopimelate and Lys, to Met, and to Thr; the NADP-dependent reductive dephosphorylation of L-aspartyl phosphate to L-aspartate-semialdehyde. In bacteria and fungi, ASD is a protein of about 40 Kd (340 to 370 residues) whose sequence is not extremely well conserved [1]. A conserved cysteine residue has been implicated as important for the catalytic activity [2]. The region of conservation around the active site residue is too small to be used as signature pattern. Another more conserved region, located in the last third of the sequence, and which contains both a conserved cysteine

20 as well as an histidine has been used instead.

25

Consensus pattern: [LIVM]-[SADN]-x(2)-C-x-R-[LIVM]-x(4)-[GSC]-H-[STA

[1] Baril C., Richaud C., Fourni E., Baranton G., Saint Girons I. J. Gen. Microbiol. 138:47-53(1992).[2] Karsten W.E., Viola R.E. Biochim. Biophys. Acta 1121:234-238(1992).

30

N-acetyl-gamma-glutamyl-phosphate reductase active site

N-acetyl-gamma-glutamyl-phosphate reductase (EC 1.2.1.38) (AGPR) [1,2] is the enzyme that catalyzes the third step in the biosynthesis of arginine from glutamate, the NADP-dependent reduction of N-acetyl-5-glutamyl phosphate into N-acetylglutamate 5-semialdehyde. In bacteria it is a monofunctional protein of 35 to 38 Kd (gene argC) while in

fungi it is part of a bifunctional mitochondrial enzyme (gene ARG5,6, arg11 orarg-6) which contains a N-terminal acetylglutamate kinase (EC 2.7.2.8) domain and a C-terminal AGPR domain. In the *Escherichia coli* enzyme, a cysteine has been shown to be implicated in the catalytic activity, the region around this residue is well conserved and can be used as a

5 signature pattern.

Consensus pattern: [LIVM]-[GSA]-x-P-G-C-[FY]-[AVP]-T-[GA]-x(3)-[GTAC]-[LIVM]- x-
P [C is the active site residue]

[1] Ludovice M., Martin J.F., Carrachas P., Liras P. J. Bacteriol. 174:4606-4613(1992).[2]
Gessert S.F., Kim J.H., Nargang F.E., Weiss R.L. J. Biol. Chem. 269:8189-8203(1994).

10

609. Sialyltransferase family,
Number of members: 18

15

610. SpoU rRNA Methylase family

This family of proteins probably use S-AdoMet. Number of members: 58

[1] SpoU protein of *Escherichia coli* belongs to a new family of putative rRNA methylases.

Koonin EV, Rudd KE; Nucleic Acids Res 1993;21:5519-5519. [2] The spoU gene of
20 *escherichia coli*, the fourth gene of the spoT operon, is essential for tRNA (Gm18) 2' methyltransferase activity. Persson BC, Jager G, Gustafsson C; Nucleic Acids Res 1997;25:4093-4097.

25 611. Stathmin family signatures

Stathmin [1] (from the Greek 'stathmos' which means relay), is an ubiquitous intracellular protein, present in a variety of phosphorylated forms and which serves as a relay for diverse second messenger pathways. Its expression and phosphorylation are regulated throughout development and in response to extracellular signals regulating cell proliferation,

30 differentiation and function. Stathmin is a highly conserved protein of 149 amino acid residues. Structurally, it consists of an N-terminal domain of about 45 residues followed by a 78 residue alpha-helical domain consisting of a heptad repeat coiled coil structure and a C-terminal domain of 25 residues. Protein SCG10 is a neuron-specific, membrane-associated protein that accumulates in the growth cones of developing neurons. It is highly similar in its

sequence to stathmin, but differs in that it contains an additional N-terminal hydrophobic segment of 32 residues which is probably responsible for its interaction with membranes.

Xenopus protein XB3 is also evolutionary related to stathmin and also contains an additional N-terminal hydrophobic domain [2]. A conserved decapeptide which ends with the first three

- 5 residues of the coiled coil domain and a second pattern that corresponds to part of the central region of the coiled coil have been selected as signatures for proteins of the stathmin family.

Consensus pattern: P-[KRO]-[KR](2)-[DE]-x-S-L-[EG]-E-

Consensus pattern: A-E-K-R-E-H-E-[KR]-E-

[1] Sobel A. Trends Biochem. Sci. 16:301-305(1991), [2] Maucuer A., Moreau J., Mechali

- 10 M., Sobel A. J. Biol. Chem. 268:16420-16429(1993).

612. SUA5/yciO/yrnC family signature. The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast protein SUA5. - Escherichia coli

- 15 hypothetical protein yciO and HI1198, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein yrdC and HI0656, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein ywlC. - Mycobacterium leprae hypothetical protein in rfe-hemK intergenic region. - Methanococcus jannaschii hypothetical protein MJ0062. These are proteins of from 20 to 46 Kd which contain a number of conserved
- 20 regions in their N-terminal section. They can be picked up in the database by the following pattern.

Consensus pattern: [LIVMTA](3)-[LIVMFYC]-[PG]-T-[DE]-[STA]-x-[FY]-[GA]-[LIVM]-[GS]-

- 25

[1] Bairoch A., Rudd K.E., Robison K. Unpublished observations (1995).

613. Sucrose synthase

- 30 Sucrose synthases catalyse the synthesis of sucrose from UDP-glucose and fructose. This family includes the bulk of the sucrose synthase protein. However the carboxyl terminal region of the sucrose synthases belongs to the glycosyl transferase family Glycosyl transf 1.

614. Sulfotransferase proteins

Number of members: 59

5 615. Synaptophysin / synaptoporin signature

Synaptophysin and synaptoporin [1] are structurally related proteins, found in the membrane of synaptic vesicles, which may function as ionic or solute channels. These two glycoproteins seem to span the membrane four times. Both their N- and C-termini sequences seem to be cytoplasmically located. As a signature pattern for this family of proteins, a highly conserved
10 region located in the beginning of the first intravesicular loop just after the first transmembrane domain has been selected. This region contains a cysteine residue that may be involved in a disulfide bond.

Consensus pattern: L-S-V-[DE]-C-x-N-K-T [C may be involved in a disulfide bond
[1] Knaus P., Marqueze-Pouey B., Scherer H., Betz H. Neuron 5:453-462(1990).

15 616. Syndecans signature

Syndecans [1,2] (from the greek syndein; to bind together) are a family of transmembrane heparan sulfate proteoglycans which are implicated in the binding of extracellular matrix
20 components and growth factors. Syndecans bind a variety of molecules via their heparan sulfate chains and can act as receptors or as co-receptors. Structurally, these proteins consist of four separate domains: a) A signal sequence; b) An extracellular domain (ectodomain) of variable length and whose sequence is not evolutionary conserved in the various forms of syndecans. The ectodomain contains the sites of attachment of the heparan sulfate
25 glycosaminoglycan side chains; c) A transmembrane region; d) A highly conserved cytoplasmic domain of about 30 to 35 residues which could interact with cytoskeletal proteins. The proteins known to belong to this family are: - Syndecan 1. - Syndecan 2 or fibroglycan. - Syndecan 3 or neuroglycan or N-syndecan. - Syndecan 4 or amphiglycan or ryudocan. - Drosophila syndecan. - Caenorhabditis elegans probable syndecan (F57C7.3). The
30 signature pattern that has been developed for syndecans starts with the last residue of the transmembrane region and includes the first 10 residues of the cytoplasmic domain. This region, which contains four basic residues, could act as a stop transfer site.

Consensus pattern: [FY]-R-[IM]-[KR]-K(2)-D-E-G-S-Y

[1] Bernfield M., Kokenyesi R., Kato M., Hinkes M.T., Spring J., Gallo R.L., Lose E.J.
Annu. Rev. Cell Biol. 8:365-393(1992).[2] David G. FASEB J. 7:1023-1030(1993).

5 617. Syntaxin / epimorphin family signature

The following proteins have been shown to be evolutionary related [1,2,3]: - Epimorphin (or syntaxin 2), a mammalian mesenchymal protein which plays an essential role in epithelial morphogenesis. - Syntaxin 1A (also known as antigen HPC-1) and syntaxin 1B which are synaptic proteins which may be involved in docking of synaptic vesicles at presynaptic active zones. - Syntaxin 3. - Syntaxin 4, which is potentially involved in docking of synaptic vesicles at presynaptic active zones. - Syntaxin 5, which mediates endoplasmic reticulum to golgi transport. - Syntaxin 6, which is involved in intracellular vesicle trafficking. - Syntaxin 7. - Yeast PEP12 (or VPS6) which is required for the transport of proteases to the vacuole. - Yeast SED5 which is required for the fusion of transport vesicles with the Golgi complex. - Yeast SSO1 and SSO2 which are required for vesicle fusion with the plasma membrane. - Yeast VAM3, which is required for vacuolar assembly. - Arabidopsis thaliana protein KNOLLE which may be involved in cytokinesis. - Caenorhabditis elegans hypothetical proteins F35C8.4, F48F7.2, F55A11.2 and T01B11.3. The above proteins share the following characteristics: a size ranging from 30 Kd to 40 Kd; a C-terminal extremity which is highly hydrophobic and is probably involved in anchoring the protein to the membrane; a central, well conserved region, which seems to be in a coiled-coil conformation. The pattern specific for this family is based on the most conserved region of the coiled coil domain.

Consensus pattern: [RQ]-x(3)-[LIVMA]-x(2)-[LIVM]-[ESH]-x(2)-[LIVMT]-x-[DEVMT]-[LIVM]-x(2)-[LIVM]-[FS]-x(2)-[LIVM]-x(3)-[LIVT]-x(2)-Q- [GADEQ]-x(2)-[LIVM]-[DNQT]-x-[LIVMF]-[DESV]-x(2)-[LIVM]

[1] Bennett M.K., Garcia-Arraras J.E., Elferink L.A., Peterson K., Fleming A.M., Hazuka C.D., Scheller R.H. *Cell* 74:863-873(1993).[2] Spring J., Kato M., Bernfield M. *Trends Biochem. Sci.* 18:124-125(1993).[3] Pelham H.R.B. *Cell* 73:425-426(1993).

618. Sm protein

The U1, U2, U4/U6, and U5 small nuclear ribonucleoprotein particles (snRNPs) involved in pre-mRNA splicing contain seven Sm proteins (B/B', D1, D2, D3, E, F and G) in common, which

assemble around the Sm site present in four of the major spliceosomal small nuclear RNAs. These proteins contain a common sequence motif in two segments, Sm1 and Sm2, separated by a short variable linker.

[1] Hermann H, Fabrizio P, Raker VA, Foulaki K, Hornig H, Brahms H, Luhrmann R EMBO J 1995;14:2076-2088. [2] Kambach C, Walke S, Young R, Avis JM, de la Fortelle E, Raker VA, Luhrmann R, Li J, Nagai K; Cell 1999;96:375-387.

619. Skp1 family

[1] Stebbins CE, Kaelin WG Jr, Pavletich NP; Science 1999;284:455-461.

620. Protein secY signatures

The eubacterial secY protein [1] plays an important role in protein export. It interacts with the signal sequences of secretory proteins as well as with two other components of the protein translocation system: secA and secE. SecY is an integral plasma membrane protein of 419 to 492 amino acid residues that apparently contains ten transmembrane segments. Such a structure probably confers to secY a 'translocator' function, providing a channel for periplasmic and outer-membrane precursor proteins. Homologs of secY are found in archaeobacteria [2]. SecY is also encoded in the chloroplast genome of some algae [3] where it could be involved in a prokaryotic-like protein export system across the two membranes of the chloroplast endoplasmic reticulum (CER) which is present in chromophyte and cryptophyte algae. Two signature patterns have been developed for secY proteins. The first corresponds to the second transmembrane region, which is the most conserved section of these proteins. The second spans the C-terminal part of the fourth transmembrane region, a short intracellular loop, and the N-terminal part of the fifth transmembrane region.

Consensus pattern: [GST]-[LIVMF](2)-x-[LIVM]-G-[LIVM]-x-P-[LIVMFY](2)-x-[AS]-[GSTQ]-[LIVMFAT](3)-Q-[LIVMFA](2)

Consensus pattern: [LIVMFYW](2)-x-[DE]-x-[LIVMF]-[STN]-x(2)-G-[LIVMF]-[GST]-[NST]-G-x-[GST]-[LIVMF](3)

[1] Ito K. Mol. Microbiol. 6:2423-2428(1992).[2] Auer J., Spicker G., Boeck A. Biochimie 73:683-688(1991).[3] Douglas S.E. FEBS Lett. 298:93-96(1992).

- 5 621. (Seed protein) Small hydrophilic plant seed proteins signature. The following small hydrophilic plant seed proteins are structurally related: - Arabidopsis thaliana proteins GEA1 and GEA6. - Cotton late embryogenesis abundant (LEA) protein D-19. - Carrot EMB-1 protein. - Barley LEA proteins B19.1A, B19.1B, B19.3 and B19.4. - Maize late embryogenesis abundant protein Emb564. - Radish late seed maturation protein p8B6. - Rice
- 10 embryonic abundant protein Emp1. - Sunflower 10 Kd late embryogenesis abundant protein (DS10). - Wheat Em proteins. These proteins contains from 83 to 153 amino acid residues and may play a role[1,2] in equipping the seed for survival, maintaining a minimal level of hydration in the dry organism and preventing the denaturation of cytoplasmic components. They may also play a role during imbibition by controlling water uptake. As a signature
- 15 pattern, the best conserved region in the sequence of these proteins has been developed, it is a glycine-rich nonapeptide located in the N-terminal section.-

Consensus pattern: G-[EQ]-T-V-V-P-G-G-T-

- 20 [1] Dure L. III, Crouch M., Harada J., Ho T.-H. D., Mundy J., Quatrano R., Thomas T., Sung Z.R. Plant Mol. Biol. 12:475-486(1989).[2] Gaubier P., Raynal M., Hull G., Huestis G.M., Grellet F., Arenas C., Pages M., Delseny M. Mol. Gen. Genet. 238:409-418(1993).

- 25 622. Serine carboxypeptidases, active sites
All known carboxypeptidases are either metallo carboxypeptidases or
serinecarboxypeptidases. The catalytic activity of the serine carboxypeptidases, like that of
the trypsin family serine proteases, is provided by a charge relay system involving an aspartic
acid residue hydrogen-bonded to a histidine, which is itself hydrogen-bonded to a serine [1].
- 30 Proteins known to be serine carboxypeptidases are: - Barley and wheat serine
carboxypeptidases I, II, and III [2]. - Yeast carboxypeptidase Y (YSCY) (gene PRC1), a
vacuolar protease involved in degrading small peptides. - Yeast KEX1 protease, involved in
killer toxin and alpha-factor precursor processing. - Fission yeast *ssa2*, a probable
carboxypeptidase involved in degrading or processing mating pheromones [3]. - Penicillium

janthinellum carboxypeptidase S1 [4]. - *Aspergillus niger* carboxypeptidase pepF. - *Aspergillus sato*i carboxypeptidase cpdS. - Vertebrate protective protein / cathepsin A [5], a lysosomal protein which is not only a carboxypeptidase but also essential for the activity of both beta-galactosidase and neuraminidase. - Mosquito vitellogenic carboxypeptidase (VCP)

- 5 [6]. - *Naegleria fowleri* virulence-related protein Nf314 [7]. - Yeast hypothetical protein YBR139w. - *Caenorhabditis elegans* hypothetical proteins C08H9.1, F13D12.6, F32A5.3, F41C3.5 and K10B2.2. This family also includes: - Sorghum (s)-hydroxymandelonitrile lyase (hydroxynitrile lyase) (HNL) [8], an enzyme involved in plant cyanogenesis. The sequences surrounding the active site serine and histidine residues are highly conserved in all these
- 10 serine carboxypeptidases.

Consensus pattern: [LIVM]-x-[GTA]-E-S-Y-[AG]-[GS] [S is the active site residue]

Consensus pattern: [LIVF]-x(2)-[LIVSTA]-x-[IVPST]-x-[GSDNQL]-[SAGV]-[SG]-H-x-[IVAQ]-P-x(3)-[PSA] [H is the active site residue]

- [1] Liao D.I., Remington S.J. J. Biol. Chem. 265:6528-6531(1990).[2] Sorensen S.B., Svendsen I., Breddam K. Carlsberg Res. Commun. 54:193-202(1989).[3] Imai Y., Yamamoto M. Mol. Cell. Biol. 12:1827-1834(1992).[4] Svendsen I., Hofmann T., Endrizzi J., Remington J., Breddam K. FEBS Lett. 333:39-43(1993).[5] Galjart N.J., Morreau H., Willemsen R., Gillemans N., Bonten E.J., d'Azzo A. J. Biol. Chem. 266:14754-14762(1991).[6] Cho W.L., Deitsch K.W., Raikhel A.S. Proc. Natl. Acad. Sci. U.S.A. 88:10821-10824(1991).[7] Hu W.N., Kopachik W., Band R.N. Infect. Immun. 60:2418-2424(1992).[8] Wajant H., Mundry K.W., Pfizenmaier K. Plant Mol. Biol. 26:735-746(1994).[9] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).[E1]
- 20

- 25 623. Serpins signature. Serpins (SERine Proteinase INhibitors) [1,2,3,4] are a group of structurally related proteins. They are high molecular weight (400 to 500 amino acids), extracellular, irreversible serine protease inhibitors with a well defined structural-functional characteristic: a reactive region that acts as a 'bait' for an appropriate serine protease. This region is found in the C-terminal part of these proteins. Proteins which are
- 30 known to belong to the serpin family are listed below (references are only provided for recently determined sequences): - Alpha-1 protease inhibitor (alpha-1-antitrypsin, contrapsin). - Alpha-1-antichymotrypsin. - Antithrombin III. - Alpha-2-antiplasmin. - Heparin cofactor II. - Complement C1 inhibitor. - Plasminogen activator inhibitors 1 (PAI-1) and 2 (PAI-2). - Glia derived nexin (GDN) (Protease nexin I). - Protein C inhibitor. - Rat

hepatocytes SPI-1, SPI-2 and SPI-3 inhibitors. - Human squamous cell carcinoma antigen (SCCA) which may act in the modulation of the host immune response against tumor cells. - A lepidopteran protease inhibitor. - Leukocyte elastase inhibitor which, in contrast to other serpins, is an intracellular protein. - Neuroserpin [5], a neuronal inhibitor of plasminogen activators and plasmin. - Cowpox virus crmA [6], an inhibitor of the thiol protease interleukin-1B converting enzyme (ICE). CrmA is the only serpin known to inhibit a non-serine proteinase. - Some orthopoxviruses probable protease inhibitors, which may be involved in the regulation of the blood clotting cascade and/or of the complement cascade in the mammalian host. On the basis of strong sequence similarities, a number of proteins with no known inhibitory activity are said to belong to this family: - Birds ovalbumin and the related genes X and Y proteins. - Angiotensinogen; the precursor of the angiotensin active peptide. - Barley protein Z; the major endosperm albumin. - Corticosteroid binding globulin (CBG). - Thyroxine-binding globulin (TBG). - Sheep uterine milk protein (UTMP) and pig uteroferrin-associated protein (UFAP). - Hsp47, an endoplasmic reticulum heat-shock protein that binds strongly to collagen and could act as a chaperone in the collagen biosynthetic pathway [7]. - Maspin, which seems to function as a tumor suppressor [5]. - Pigment epithelium-derived factor precursor (PEDF), a protein with a strong neutrophilic activity [8]. - Ep45, an estrogen-regulated protein from *Xenopus* [9]. A signature pattern has been developed for this family of proteins, centered on a well conserved Pro-Phe sequence which is found ten to fifteen residues on the C-terminal side of the reactive bond

Consensus pattern: [LIVMFY]-x-[LIVMFYAC]-[DNQ]-[RKHQ]-[PST]-F-[LIVMFY]-[LIVMFYC]-x-[LIVMFAH]-

[1] Carrell R., Travis J. Trends Biochem. Sci. 10:20-24(1985).[2] Carrell R., Pemberton P.A., Boswell D.R. Cold Spring Harbor Symp. Quant. Biol. 52:527-535(1987).[3] Huber R., Carrell R.W. Biochemistry 28:8951-8966(1989).[4] Remold-O'Donneel E. FEBS Lett. 315:105-108(1993).[5] Osterwalder T., Contartese J., Stoeckli E.T., Kuhn T.B., Sonderegger P. EMBO J. 15:2944-2953(1996).[6] Komiyama T., Ray C.A., Pickup D.J., Howard A.D., Thornberry N.A., Peterson E.P., Salvesen G. J. Biol. Chem. 269:19331-19337(1994).[7] Clarke E., Sandwal B.D. Biochim. Biophys. Acta 1129:246-248(1992).[8] Zou Z., Anisowicz A., Neveu M., Rafidi K., Sheng S., Sager R., Hendrix M.J., Seftor E., Thor A. Science 263:526-529(1994).[9] Steele F.R., Chader G.J., Johnson L.V., Tombran-Tink J.

Proc. Natl. Acad. Sci. U.S.A. 90:1526-1530(1993).[10] Holland L.J., Suksang C., Wall A.A., Roberts L.R., Moser D.R., Bhattacharya A. J. Biol. Chem. 267:7053-7059(1992).

5 624. Sigma-54 interaction domain signatures and profile

Some bacterial regulatory proteins activate the expression of genes from promoters recognized by core RNA polymerase associated with the alternative sigma-54 factor. These have a conserved domain of about 230 residues involved in the ATP-dependent [1,2] interaction with sigma-54. This domain has been found in the proteins listed below: - *acoR* from *Alcaligenes eutrophus*, an activator of the acetoin catabolism operon *acoXABC*. - *algB* from *Pseudomonas aeruginosa*, an activator of alginate biosynthetic gene *algD*. - *dctD* from *Rhizobium*, an activator of *dctA*, the C4-dicarboxylate transport protein. - *dhaR* from *Citrobacter freundii*, a regulator of the *dha* operon for glycerol utilization. - *fhlA* from *Escherichia coli*, an activator of the formate dehydrogenase H and hydrogenase III structural genes. - *flbD* from *Caulobacter crescentus*, an activator of flagellar genes. - *hoxA* from *Alcaligenes eutrophus*, an activator of the hydrogenase operon. - *hrpS* from *Pseudomonas syringae*, an activator of *hprD* as well as other *hrp* loci involved in plant pathogenicity. - *hupR1* from *Rhodobacter capsulatus*, an activator of the [NiFe] hydrogenase genes *hupSL*. - *hydG* from *Escherichia coli* and *Salmonella typhimurium*, an activator of the hydrogenase activity. - *levR* from *Bacillus subtilis*, which regulates the expression of the levanase operon (*levDEFG* and *sacC*). - *nifA* (as well as *anfA* and *vnfA*) from various bacteria, an activator of the *nif* nitrogen-fixing operon. - *ntrC*, from various bacteria, an activator of nitrogen assimilatory genes such as that for glutamine synthetase (*glnA*) or of the *nif* operon. - *pgtA* from *Salmonella typhimurium*, the activator of the inducible phospho- glycerate transport system. - *pilR* from *Pseudomonas aeruginosa*, an activator of pilin gene transcription. - *rocR* from *Bacillus subtilis*, an activator of genes for arginine utilization - *tyrR* from *Escherichia coli*, involved in the transcriptional regulation of aromatic amino-acid biosynthesis and transport. - *wtsA*, from *Erwinia stewartii*, an activator of plant pathogenicity gene *wtsB*. - *xylR* from *Pseudomonas putida*, the activator of the *tol* plasmid xylene catabolism operon *xylCAB* and of *xylS*. - *Escherichia coli* hypothetical protein *yfhA*. - *Escherichia coli* hypothetical protein *yhgB*. About half of these proteins (*algB*, *dctD*, *flbD*, *hoxA*, *hupR1*, *hydG*, *ntrC*, *pgtA* and *pilR*) belong to signal transduction two-component systems [3] and possess a domain that can be phosphorylated by a sensor-kinase protein in their N- terminal section. Almost all of these proteins possess a helix-turn-helix DNA-binding domain in their

C-terminal section. The domain which interacts with the sigma-54 factor has an ATPase activity. This may be required to promote a conformational change necessary for the interaction [4]. The domain contains an atypical ATP-binding motif A (P-loop) as well as a form of motif B. The two ATP-binding motifs are located in the N-terminal section of the domain; signature patterns have been developed for both motifs. Other regions of the domain are also conserved. One of them, located in the C-terminal section, has been selected as a third signature pattern.

Consensus pattern: [LIVMFY](3)-x-G-[DEQ]-[STE]-G-[STAV]-G-K-x(2)-[LIVMFY]

Consensus pattern: [GS]-x-[LIVMF]-x(2)-A-[DNEQASH]-[GNEK]-G-[STIM]-

10 [LIVMFY](3)-[DE]-[EK]-[LIVM]

Consensus pattern: [FYW]-P-[GS]-N-[LIVM]-R-[EQ]-L-x-[NHAT]

[1] Morrett E., Segovia L. J. Bacteriol. 175:6067-6074(1993).[2] Austin S., Kundrot C., Dixon R. Nucleic Acids Res. 19:2281-2287(1991).[3] Albright L.M., Huala E., Ausubel F.M. Annu. Rev. Genet. 23:311-336(1989).[4] Austin S., Dixon R. EMBO J. 11:2219-2228(1992).

625. Sigma-70 factors family signatures

- Sigma factors [1] are bacterial transcription initiation factors that promote the attachment of the core RNA polymerase to specific initiation sites and are then released. They alter the specificity of promoter recognition. Most bacteria express a multiplicity of sigma factors. Two of these factors, sigma-70 (gene *rpoD*), generally known as the major or primary sigma factor, and sigma-54 (gene *rpoN* or *ntxA*) direct the transcription of a wide variety of genes. The other sigma factors, known as alternative sigma factors, are required for the transcription of specific subsets of genes. With regard to sequence similarity, sigma factors can be grouped into two classes: the sigma-54 and sigma-70 families. The sigma-70 family includes, in addition to the primary sigma factor, a wide variety of sigma factors, some of which are listed below: - *Bacillus* sigma factors involved in the control of sporulation-specific genes: sigma-E (sigE or spoIIGB), sigma-F (sigF or spoIAC), sigma-G (sigG or spoIIG), sigma-H (sigH or spo0C) and sigma-K (sigK or spoIVCB/spoIIIC). - *Escherichia coli* and related bacteria sigma-32 (gene *rpoH* or *htpR*) involved in the expression of heat shock genes. - *Escherichia coli* and related bacteria sigma-27 (gene *fliA*) involved in the expression of the flagellin gene. - *Escherichia coli* sigma-S (gene *rpoS* or *katF*) which seems to be involved in the expression of genes required for protection against external stresses. - *Myxococcus xanthus* sigma-B

(sigB) which is essential for the late-stage differentiation of that bacteria. Alignments of the sigma-70 family permit the identification of four regions of high conservation [2,3]. Each of these four regions can in turn be subdivided into a number of sub-regions. Signature patterns based on the two best-conserved sub-regions have been developed. The first pattern

- 5 corresponds to sub-region 2.2; the exact function of this sub-region is not known although it could be involved in the binding of the sigma factor to the core RNA polymerase. The second pattern corresponds to sub-region 4.2 which seems to harbor a DNA-binding 'helix-turn-helix' motif involved in binding the conserved -35 region of promoters recognized by the major sigma factors. The second pattern starts one residue before the N-terminal extremity of the
- 10 HTH region and ends six residues after its C-terminal extremity.

Consensus pattern: [DE]-[LIVMF](2)-[HEQS]-x-G-x-[LIVMFA]-G-L-[LIVMFYE]-x-[GSAM]-[LIVMAP]

Consensus pattern: [STN]-x(2)-[DEQ]-[LIVM]-[GAS]-x(4)-[LIVMF]-[PSTG]-x(3)-[LIVMA]-x-[NQR]-[LIVMA]-[EQH]-x(3)-[LIVMFW]-x(2)-[LIVM]

- 15 [1] Hermann J.D., Chamberlin M.J. Annu. Rev. Biochem. 57:839-872(1988). [2] Gribskov M., Burgess R.R. Nucleic Acids Res. 14:6745-6763(1986). [3] Lonetto M.A., Gribskov M., Gross C.A. J. Bacteriol. 174:3843-3849(1992). [4] Lonetto M.A., Brown K.L., Rudd K.E., Buttner M.J. Proc. Natl. Acad. Sci. U.S.A. 91:7573-7577(1994).

- 20 626. Signal carboxyl-terminal domain. 430 members.

627. Signal peptidases I signatures

- 25 Signal peptidases (SPases) [1] (also known as leader peptidases) remove the signal peptides from secretory proteins. In prokaryotes three types of SPases are known: type I (gene *lepB*) which is responsible for the processing of the majority of exported pre-proteins; type II (gene *lsp*) which only process lipoproteins, and a third type involved in the processing of pili subunits. SPase I is an integral membrane protein that is anchored in the cytoplasmic
- 30 membrane by one (in *B. subtilis*) or two (in *E. coli*) N-terminal transmembrane domains with the main part of the protein protruding in the periplasmic space. Two residues have been shown [2,3] to be essential for the catalytic activity of SPase I: a serine and an lysine. SPase I is evolutionary related to the yeast mitochondrial inner membrane protease subunit 1 and 2 (genes *IMP1* and *IMP2*) which catalyze the removal of signal peptides required for the

targeting of proteins from the mitochondrial matrix, across the inner membrane, into the inter-membrane space [4]. In eukaryotes the removal of signal peptides is effected by an oligomeric enzymatic complex composed of at least five subunits: the signal peptidase complex (SPC). The SPC is located in the endoplasmic reticulum membrane. Two components of mammalian SPC, the 18 Kd (SPC18) and the 21 Kd (SPC21) subunits as well as the yeast SEC11 subunit have been shown [5] to share regions of sequence similarity with prokaryotic SPases I and yeast IMP1/IMP2. Three signature patterns for these proteins have been developed. The first signature contains the putative active site serine, the second signature contains the putative active site lysine which is not conserved in the SPC subunits, and the third signature corresponds to a conserved region of unknown biological significance which is located in the C-terminal section of all these proteins.

Consensus pattern: [GS]-x-S-M-x-[PS]-[AT]-[LF] [S is an active site residue]

Consensus pattern: K-R-[LIVMSTA](2)-G-x-[PG]-G-[DE]-x-[LIVM]-x-[LIVMFY] [K is an active site residue]

Consensus pattern: [LIVMFYW](2)-x(2)-G-D-[NH]-x(3)-[SND]-x(2)-[SG]

[1] Dalbey R.E., von Heijne G. Trends Biochem. Sci. 17:474-478(1992).[2] Sung M., Dalbey R.E. J. Biol. Chem. 267:13154-13159(1992).[3] Black M.T. J. Bacteriol. 175:4957-4961(1993).[4] Nunnari J., Fox T.D., Walter P. Science 262:1997-2004(1993).[5] van Dijk J.M., de Jong A., Vehmaanpera J., Venema G., Bron S. EMBO J. 11:2819-2828(1992).[6] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).[E1]

628. (sodcu) Copper/Zinc superoxide dismutase signatures

Copper/Zinc superoxide dismutase (SODC) [1] is one of the three forms of an enzyme that catalyzes the dismutation of superoxide radicals. SODC binds one atom each of zinc and copper. Various forms of SODC are known: acytoplasmic form in eukaryotes, an additional chloroplast form in plants, an extracellular form in some eukaryotes, and a periplasmic form in prokaryotes. The metal binding sites are conserved in all the known SODC sequences [2]. Two signature patterns have been derived for this family of enzymes: the first one contains two histidine residues that bind the copper atom; the second one is located in the C-terminal section of SODC and contains a cysteine which is involved in a disulfide bond.

Consensus pattern: [GA]-[IMFAT]-H-[LIVF]-H-x(2)-[GP]-[SDG]-x-[STAGDE] [The two H's are copper ligands]

Consensus pattern: G-[GN]-[SGA]-G-x-R-x-[SGA]-C-x(2)-[IV] [C is involved in a disulfide bond]

- [1] Bannister J.V., Bannister W.H., Rotilio G. *CRC Crit. Rev. Biochem.* 22:111-154(1987).
[2] Smith M.W., Doolittle R.F. *J. Mol. Evol.* 34:175-184(1992).

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629. (sodfe) Manganese and iron superoxide dismutases signature

Manganese superoxide dismutase (SODM) [1] is one of the three forms of an enzyme that catalyzes the dismutation of superoxide radicals. The four ligands of the manganese atom are conserved in all the known SODM sequences. These metal ligands are also conserved in the related iron form of superoxide dismutases [2,3]. A short conserved region which includes two of the four ligands: an aspartate and a histidine has been selected as a signature.

- Consensus pattern: D-x-W-E-H-[STA]-[FY](2) [D and H are manganese/iron ligands]
[1] Bannister J.V., Bannister W.H., Rotilio G. *CRC Crit. Rev. Biochem.* 22:111-154(1987).
[2] Parker M.W., Blake C.C.F. *FEBS Lett.* 229:377-382(1988).
[3] Smith M.W., Doolittle R.F. *J. Mol. Evol.* 34:175-184(1992).

630. Spectrin repeat

Spectrin repeats are found in several proteins involved in cytoskeletal structure. These include spectrin, alpha-actinin and dystrophin. The sequence repeat used in this family is taken from the structural repeat in reference [2]. The spectrin repeat forms a three helix bundle. The second helix is interrupted by proline in some sequences.

25 Number of members: 898

- [1] Actin-binding proteins. 1: Spectrin super family. Hartwig JH; *Protein Profile* 1995;2:732-732. [2] Crystal structure of the repetitive segments of spectrin. Yan Y, Winograd E, Viel A, Cronin T, Harrison SC, Branton D; *Science* 1993;262:2027-2030.

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631. (subtilase) Streptomyces subtilisin-type inhibitors signature

Bacteria of the Streptomyces family produce a family of proteinase inhibitors[1] characterized by their strong activity toward subtilisin. They are collectively known as SSI's: Streptomyces Subtilisin Inhibitors. Some SSI's also inhibit trypsin or chymotrypsin. In their

mature secreted form, SSI's are proteins of about 110 residues with two conserved disulfide bonds. +-----+ +-----+ |||

xxxxxxxxxxxxCxxxxxxxxCxxxxxxxxCx#xxxxxxxxxxxxCxxxxxx *****'C':

conserved cysteine involved in a disulfide bond. #: active site residue. '*': position of the

5 pattern.

Consensus pattern: C-x-P-x(2,3)-G-x-H-P-x(4)-A-C-[ATD]-x-L [The two C's are involved in a disulfide bond]

[1] Taguchi S., Kojima S., Terabe M., Miura K.-I., Momose H. Eur. J. Biochem. 220:911-918(1994).

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632. Sugar transport proteins signatures

In mammalian cells the uptake of glucose is mediated by a family of closely related transport proteins which are called the glucose transporters [1,2,3]. At least seven of these transporters

15 are currently known to exist (in Human they are encoded by the GLUT1 to GLUT7 genes). These integral membrane proteins are predicted to comprise twelve membrane

spanning domains. The glucose transporters show sequence similarities [4,5] with a number of other sugar or metabolite transport proteins listed below (references are only provided for recently determined sequences). - *Escherichia coli* arabinose-proton symport (araE). -

20 *Escherichia coli* galactose-proton symport (galP). - *Escherichia coli* and *Klebsiella pneumoniae* citrate-proton symport (also known as citrate utilization determinant) (gene cit).

- *Escherichia coli* alpha-ketoglutarate permease (gene kgtP). - *Escherichia coli* proline/betaine transporter (gene proP) [6]. - *Escherichia coli* xylose-proton symport (xylE). - *Zymomonas mobilis* glucose facilitated diffusion protein (gene glf). - Yeast high and low

25 affinity glucose transport proteins (genes SNF3, HXT1 to HXT14). - Yeast galactose transporter (gene GAL2). - Yeast maltose permeases (genes MAL3T and MAL6T). - Yeast myo-inositol transporters (genes ITR1 and ITR2). - Yeast carboxylic acid transporter protein homolog JEN1. - Yeast inorganic phosphate transporter (gene PHO84). - *Kluyveromyces lactis* lactose permease (gene LAC12). - *Neurospora crassa* quinate transporter (gene Qa-y),

30 and *Emericella nidulans* quinate permease (gene qutD). - *Chlorella* hexose carrier (gene HUP1). - *Arabidopsis thaliana* glucose transporter (gene STP1). - Spinach sucrose transporter. - *Leishmania donovani* transporters D1 and D2. - *Leishmania enriettii* probable transport protein (LTP). - Yeast hypothetical proteins YBR241c, YCR98c and YFL040w. - *Caenorhabditis elegans* hypothetical protein ZK637.1. - *Escherichia coli* hypothetical proteins

yaBE, ydJE and yhJE. - Haemophilus influenzae hypothetical proteins HI0281 and HI0418. - Bacillus subtilis hypothetical proteins yxbC and yxdF. It has been suggested [4] that these transport proteins have evolved from the duplication of an ancestral protein with six transmembrane regions, this hypothesis is based on the conservation of two G-R-[KR] motifs.

- 5 The first one is located between the second and third transmembrane domains and the second one between transmembrane domains 8 and 9. Two patterns have been developed to detect this family of proteins. The first pattern is based on the G-R-[KR] motif; but because this motif is too short to be specific to this family of proteins, a pattern from a larger region centered on the second copy of this motif was derived. The second pattern is based on a
 - 10 number of conserved residues which are located at the end of the fourth transmembrane segment and in the short loop region between the fourth and fifth segments.
- Consensus pattern: [LIVMSTAG]-[LIVMFSAG]-x(2)-[LIVMSA]-[DE]-x-[LIVMFYWA]-G-R-[RK]-x(4,6)-[GSTA]
- Consensus pattern: [LIVMF]-x-G-[LIVMFA]-x(2)-G-x(8)-[LIFY]-x(2)-[EQ]-x(6)-[RK]
- 15 [1] Silverman M. Annu. Rev. Biochem. 60:757-794(1991). [2] Gould G.W., Bell G.I. Trends Biochem. Sci. 15:18-23(1990). [3] Baldwin S.A. Biochim. Biophys. Acta 1154:17-49(1993). [4] Maiden M.C.J., Davis E.O., Baldwin S.A., Moore D.C.M., Henderson P.J.F. Nature 325:641-643(1987). [5] Henderson P.J.F. Curr. Opin. Struct. Biol. 1:590-601(1991). [6] Culham D.E., Lasby B., Marangoni A.G., Milner J.L., Steer B.A., van Nues R.W., Wood
 - 20 J.M. J. Mol. Biol. 229:268-276(1993).

633. Synaptobrevin signature

- Synaptobrevin [1] is an intrinsic membrane protein of small synaptic vesicles whose function
- 25 is not yet known, but which is highly conserved in mammals, electric ray (where it is known as VAMP-1), Drosophila and yeast [2]. In yeast there are two closely related forms of synaptobrevin (genes SNC1 and SNC2) while in mammals there is at least 4 (genes SYB1, SYB2, SYB3 and SYBL1). Structurally synaptobrevin consists of a N-terminal cytoplasmic domain of from 90 to 110 residues, followed by a transmembrane region, and then by a short
 - 30 (from 2 to 22 residues) C-terminal intravesicular domain. As a signature pattern for synaptobrevin, a highly conserved stretch of residues located in the central part of the sequence was selected.

Consensus pattern: N-[LIVM]-[DENS]-[KL]-V-x-[DEQ]-R-x(2)-[KR]-[LIVM]-[STDE]-x-[LIVM]-x-[DE]-[KR]-[TA]-[DE]

[1] Suedhof T.C., Baumert M., Perin M.S., Jahn R. Neuron 2:1475-1481(1989).[2] Gerst J.E., Rodgers L., Riggs M., Wigler M. Proc. Natl. Acad. Sci. U.S.A. 89:4338-4342(1992).

634. TBC domain. Identification of a TBC domain in GYP6_YEAST and GYP7_YEAST, which are GTPase activator proteins of yeast Ypt6 and Ypt7, imply that these domains are GTPase activator proteins of Rab-like small GTPases. Number of members: 55

[1] Medline: 96032578. Molecular cloning of a cDNA with a novel domain present in the tre-2 oncogene and the yeast cell cycle regulators BUB2 and cdc16. Richardson PM, Zon LI; Oncogene 1995;11:1139-1148.

[2]Medline: 97398935. A shared domain between a spindle assembly checkpoint protein and Ypt/Rab-specific GTPase-activators. Neuwald AF; Trends Biochem Sci 1997;22:243-244.

635. Transcription factor TFIID repeat signature (TBP)

Transcription factor TFIID (or TATA-binding protein, TBP) [1,2] is a general factor that plays a major role in the activation of eukaryotic genes transcribed by RNA polymerase II. TFIID binds specifically to the TATA box promoter element which lies close to the position of transcription initiation. There is a remarkable degree of sequence conservation of a C-terminal domain of about 180 residues in TFIID from various eukaryotic sources. This region is necessary and sufficient for TATA box binding. The most significant structural feature of this domain is the presence of two conserved repeats of a 77 amino-acid region. The intramolecular symmetry generates a saddle-shaped structure that sits astride the DNA [3]. Drosophila TRF (TBP-related factor) [4] is a sequence-specific transcription factor that also binds to the TATA box and is highly similar to TFIID. Archaeobacteria also possess a TBP homolog [5]. A signature pattern that spans the last 50 residues of the repeated region has been derived.-

Consensus pattern: Y-x-P-x(2)-[IF]-x(2)-[LIVM](2)-x-[KRH]-x(3)-P-[RKQ]-x(3)-L-[LIVM]-F-x-[STN]-G-[KR]-[LIVM]-x(3)-G-[TAGL]-[KR]-x(7)-[AGC]-x(7)-[LIVM] [1] Hoffmann A., Sinn E., Yamamoto T., Wang J., Roy A., Horikoshi M., Roeder R.G.

Nature 346:387-390(1990).[2] Gash A., Hoffmann A., Horikoshi M., Roeder R.G., Chua N.-H. Nature 346:390-394(1990).[3] Nikolov D.B., Hu S.-H., Lin J., Gash A., Hoffmann A., Horikoshi M., Chua N.-H., Roeder R.G., Burley S.K. Nature 360:40-46(1992).[4] Crowley T.E., Hoey T., Liu J.-K., Jan Y.N., Jan L.Y., Tjian R. Nature 361:557-561(1993).[5] Marsh

T.L., Reich C.I., Whitelock R.B., Olsen G.J. Proc. Natl. Acad. Sci. U.S.A. 91:4180-4184(1994).

5 636. Translationally controlled tumor protein signatures (TCTP)

Mammalian translationally controlled tumor protein (TCTP) (or P23) is a protein which has been found to be preferentially synthesized in cells during the early growth phase of some types of tumor [1,2], but which is also expressed in normal cells. The physiological function of TCTP is still not known. It is a hydrophilic protein of 18 to 20 Kd. Close homologs have been found in plants [3], earthworm [4], *Caenorhabditis elegans* (F52H2.11), Hydra, budding yeast (YKL056c) [5] and fission yeast (SpAC1F12.02c). Two of the best conserved regions have been selected as signature patterns for TCTP.

Consensus pattern: [IFA]-[GA]-[GAS]-N-[PAK]-S-[GA]-E-[GDE]-[PAGE]-[DEQGA]

Consensus pattern: [FLVH]-[FY]-[IVCT]-G-E-x-[MA]-x(2,5)-[DEN]-[GAST]-x-[LV]-[AV]-x(3)-[FYW]

[1] Boehm H., Beendorf R., Gaestel M., Gross B., Nuernberg P., Kraft R., Otto A., Bielka H. Biochem. Int. 19:277-286(1989).[2] Makrides S., Chitpatima S.T., Bandyopadhyay R., Brawerman G. Nucleic Acids Res. 16:2350-2350(1988).[3] Pay A., Heberle-Bors E., Hirt H. Plant Mol. Biol. 19:501-503(1992).[4] Stuerzenbaum S.R., Kille P., Morgan A.J. Biochim. Biophys. Acta 1398:294-304(1998).[5] Rasmussen S.W. Yeast 10:S63-S68(1994).

637. TFIIS zinc ribbon domain signature

Transcription factor S-II (TFIIS) [1] is a eukaryotic protein necessary for efficient RNA polymerase II transcription elongation, past template-encoded pause sites. TFIIS shows DNA-binding activity only in the presence of RNA polymerase II. It is a protein of about 300 amino acids whose sequence is highly conserved in mammals, *Drosophila*, yeast (where it was first known as PPR2, a transcriptional regulator of URA4, and then as DST1, the DNA strand transfer protein alpha [2]) and in the archaeobacteria *Sulfolobus acidocaldarius* [3]. This family also includes the eukaryotic and archeobacterial RNA polymerase subunits of the 15 Kd / M family (see <PDOC00790>) as well as the following viral proteins: - Vaccinia virus RNA polymerase 30 Kd subunit (rpo30) [4]. - African swine fever virus protein I243L [5]. The best conserved region of all these proteins contains four cysteines that bind a zinc ion and fold in a conformation termed a 'zinc ribbon' [6]. Besides these cysteines, there are a

number of other conserved residues which can be used to help define a specific pattern for this type of domain.

Consensus pattern: C-x(2)-C-x(9)-[LIVMQSAR]-[QH]-[STQL]-[RA]-[SACR]-x-[DE]-[DET]-[PGSEA]-x(6)-C-x(2,5)-C-x(3)-[FW] [The four C's are zinc ligands]

- 5 [1] Hirashima S., Hirai H., Nakanishi Y., Natori S. J. Biol. Chem. 263:3858-3863(1988).[2] Kipling D., Kearsley S.E. Nature 353:509-509(1991).[3] Langer D., Zillig W. Nucleic Acids Res. 21:2251-2251(1993).[4] Ahn B.-Y., Gershon P.D., Jones E.V., Moss B. Mol. Cell. Biol. 10:5433-5441(1990).[5] Rodriguez J.M., Salas M.L., Vinuela E. Virology 186:40-52(1992).[6] Qian X., Jeon C., Yoon H., Agarwal K., Weiss M.A. Nature 365:277-279(1993).

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638. Tetrahydrofolate dehydrogenase/cyclohydrolase signatures (THF DHG CYH)

Enzymes that participate in the transfer of one-carbon units are involved in various biosynthetic pathways. In many of these processes the transfers of one-carbon units mediated by the coenzyme tetrahydrofolate (THF). Various reactions generate one-carbon derivatives of THF which can be interconverted between different oxidation states by formyltetrahydrofolate synthetase(EC 6.3.4.3), methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5 or EC 1.5.1.15) and methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9). The dehydrogenase and cyclohydrolase activities are expressed by a variety of multifunctional enzymes: - Eukaryotic C-1-tetrahydrofolate synthase (C1-THF synthase), which catalyzes all three reactions described above. Two forms of C1-THF synthases are known [1], one is located in the mitochondrial matrix, while the second one is cytoplasmic. In both forms the dehydrogenase/cyclohydrolase domain is located in the N-terminal section of the 900 amino acid protein and consists of about 300 amino acid residues. The C1-THF synthases are NADP- dependent. - Eukaryotic mitochondrial bifunctional dehydrogenase/cyclohydrolase [2]. This is an homodimeric NAD-dependent enzyme of about 300 amino acid residues. - Bacterial folD [3]. FolD is an homodimeric bifunctional NADP-dependent enzyme of about 290 amino acid residues. The sequence of the dehydrogenase/cyclohydrolase domain is highly conserved in all forms of the enzyme. Two conserved regions have been selected as signature patterns. The first one is located in the N-terminal part of these enzymes and contains three acidic residues. The second pattern is a highly conserved sequence of 9 amino acids which is located in the C-terminal section.

Consensus pattern: [EQ]-x-[EQK]-[LIVM](2)-x(2)-[LIVM]-x(2)-[LIVMY]-N-x-[DN]- x(5)-[LIVMF](3)-Q-L-P-[LV]

Consensus pattern: P-G-G-V-G-P-[MF]-T-[IV]

[1] Shannon K.W., Rabinowitz J.C. J. Biol. Chem. 263:7717-7725(1988).[2] Belanger C., Mackenzie R.E. J. Biol. Chem. 264:4837-4843(1989).[3] d'Ari L., Rabinowitz J.C. J. Biol. Chem. 266:23953-23958(1991).

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639. Triosephosphate isomerase active site (TIM)

Triosephosphate isomerase (EC 5.3.1.1) (TIM) [1] is the glycolytic enzyme that catalyzes the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

10 TIM plays an important role in several metabolic pathways and is essential for efficient energy production. It is a dimer of identical subunits, each of which is made up of about 250 amino-acid residues. A glutamic acid residue is involved in the catalytic mechanism [2]. The sequence around the active site residue is perfectly conserved in all known TIM's and can be used as a signature pattern for this type of enzyme.

15 Consensus pattern: [AV]-Y-E-P-[LIVM]-W-[SA]-I-G-T-[GK] [E is the active site residue]
[1] Lolis E., Alber T., Davenport R.C., Rose D., Hartman F.C., Petsko G.A. Biochemistry 29:6609-6618(1990).[2] Knowles J.R. Nature 350:121-124(1991).

20 640. Thymidine kinase cellular-type signature (TK)

Thymidine kinase (TK) (EC 2.7.1.21) is an ubiquitous enzyme that catalyzes the ATP-dependent phosphorylation of thymidine. A comparison of TK sequences has shown [1,2,3] that there are two different families of TK. One family groups together TK from herpes viruses as well as cellular thymidylate kinases, while the second family currently consists of
25 TK from the following sources: - Vertebrates. - Bacterial. - Bacteriophage T4. - Pox viruses. - African swine fever virus (ASF). - Fish lymphocystis disease virus (FLDV). A conserved region which is located in the C-terminal section of these enzymes has been selected as a signature pattern for this family of TKA.

Consensus pattern: [GA]-x(1,2)-[DE]-x-Y-x-[STAP]-x-C-[NKR]-x-[CH]-[LIVMFYWH]
30 [1] Boyle D.B., Coupar B.E.H., Gibbs A.J., Seigman L.J., Both G.W. Virology 156:355-365(1987).[2] Blasco R., Lopez-Otin C., Munoz M., Bockamp E.-O., Simon-Mateo C., Vinuela E. Virology 178:301-304(1990).[3] Robertson G.R., Whalley J.M. Nucleic Acids Res. 16:11303-11317(1988).

641. Thymidine kinase from herpesvirus (TK herpes)

[1]

Medline: 96003730

- 5 Crystal structures of the thymidine kinase from herpes simplex virus type-1 in complex with deoxythymidine and ganciclovir.

Brown DG, Visse R, Sandhu G, Davies A, Rizkallah PJ, Melitz C, Summers WC, Sanderson MR;

- 10 Nat Struct Biol 1995;2:876-881.

Number of members: 65

642. Nuclear transition protein 2 signatures (TP2)

- 15 In mammals, the second stage of spermatogenesis is characterized by the conversion of nucleosomal chromatin to the compact, non-nucleosomal and transcriptionally inactive form found in the sperm nucleus. This condensation is associated with a double-protein transition. The first transition corresponds to the replacement of histones by several spermatid-specific proteins, also called transition proteins, which are themselves replaced by protamines during the second transition. Nuclear transition protein 2 (TP2) is one of those spermatid-specific proteins. TP2 is a basic, zinc-binding protein [1] of 116 to 137 amino-acid residues. Structurally, TP2 consists of three distinct parts: a conserved serine-rich N-terminal domain of about 25 residues, a variable central domain of 20 to 50 residues which contains cysteine residues, and a conserved C-terminal domain of about 70 residues rich in lysines and arginines. Two signature patterns for TP2 have been developed: one located in the N-terminal domain, the other in the C-terminal.
- 20
- 25

Consensus pattern: H-x(3)-H-S-[NS]-S-x-P-Q-S

Consensus pattern: K-x-R-K-x(2)-E-G-K-x(2)-K-[KR]-K

[1] Baskaran R., Rao M.R.S. Biochem. Biophys. Res. Commun. 179:1491-1499(1991).

30

643. Thiamine pyrophosphate enzymes signature (TPP enzymes)

A number of enzymes require thiamine pyrophosphate (TPP) (vitamin B1) as a cofactor. It has been shown [1] that some of these enzymes are structurally related. These related TPP

enzymes are: - Pyruvate oxidase (POX) (EC 1.2.3.3) Reaction catalyzed: pyruvate + orthophosphate + O(2) + H(2)O = acetyl phosphate + CO(2) + H(2)O(2). - Pyruvate decarboxylase (PDC) (EC 4.1.1.1) Reaction catalyzed: pyruvate = acetaldehyde + CO(2). - Indolepyruvate decarboxylase (EC 4.1.1.74) [2] Reaction catalyzed: indole-3-pyruvate = indole-3-acetaldehyde + CO(2). - Acetolactate synthase (ALS) (EC 4.1.3.18) Reaction catalyzed: 2 pyruvate = acetolactate + CO(2). - Benzoylformate decarboxylase (BFD) (EC 4.1.1.7) [3] Reaction catalyzed: benzoylformate = benzaldehyde + CO(2). A conserved region which is located in their C-terminal section has been selected as a signature pattern for these enzymes.

Consensus pattern: [LIVMF]-[GSA]-x(5)-P-x(4)-[LIVMFYW]-x-[LIVMF]-x-G-D-[GSA]-[GSAC]

[1] Green J.B.A. FEBS Lett. 246:1-5(1989).[2] Koga J., Adachi T., Hidaka H. Mol. Gen. Genet. 226:10-16(1991).[3] Tsou A.Y., Ransom S.C., Gerlt J.A., Buechter D.D., Babbitt P.C., Kenyon G.L. Biochemistry 29:9856-9862(1990).

644. TPR Domain

[1]

Medline: 95397415

Tetratricopeptide repeat interactions: to TPR or not to TPR?

Lamb JR, Tugendreich S, Hieter P;
Trends Biochem Sci 1995;20:257-259.

[2]Medline: 98151343

The structure of the tetratricopeptide repeats of protein

phosphatase 5: implications for TPR-mediated protein-protein interactions.

Das AK, Cohen PW, Barford D;

EMBO J 1998;17:1192-1199.

Number of members: 621

645. Uroporphyrin-III C-methyltransferase signatures (TP methylase)

Uroporphyrin-III C-methyltransferase (EC 2.1.1.107) (SUMT) [1,2] catalyzes the transfer of two methyl groups from S-adenosyl-L-methionine to the C-2 and C-7atoms of

uroporphyrinogen III to yield precorrin-2 via the intermediate formation of precorrin-1. SUMT is the first enzyme specific to the cobalamin pathway and precorrin-2 is a common intermediate in the biosynthesis of corrinoids such as vitamin B12, siroheme and coenzyme F430. The sequences of SUMT from a variety of eubacterial and archaeobacterial species are currently available. In species such as *Bacillus megaterium* (gene *cobA*), *Pseudomonas denitrificans* (*cobA*) or *Methanobacterium ivanovii* (gene *corA*) SUMT is a protein of about 25 to 30 Kd. In *Escherichia coli* and related bacteria, the *cysG* protein, which is involved in the biosynthesis of siroheme, is a multifunctional protein composed of a N-terminal domain, probably involved in transforming precorrin-2 into siroheme, and a C-terminal domain which has SUMT activity. The sequence of SUMT is related to that of a number of *P. denitrificans* and *Salmonella typhimurium* enzymes involved in the biosynthesis of cobalamin which also seem to be SAM-dependent methyltransferases [3,4]. The similarity is especially strong with two of these enzymes: *cobI/cbiL* which encodes S-adenosyl-L-methionine--precorrin-2 methyltransferase and *cobM/cbiF* whose exact function is not known. Two signature patterns have been developed for these enzymes. The first corresponds to a well conserved region in the N-terminal extremity (called region 1 in [1,3]) and the second to a less conserved region located in the central part of these proteins (this pattern spans what are called regions 2 and 3 in [1,3]).

Consensus pattern: [LIVM]-[GS]-[STAL]-G-P-G-x(3)-[LIVMFY]-[LIVM]-T-[LIVM]-[KRHQG]-[AG]

Consensus pattern: V-x(2)-[LI]-x(2)-G-D-x(3)-[FYW]-[GS]-x(8)-[LIVF]-x(5,6)-[LIVMFYWPAC]-x-[LIVMY]-x-P-G

[1] Blanche F., Robin C., Couder M., Faucher D., Cauchois L., Cameron B., Crouzet J. J. *Bacteriol.* 173:4637-4645(1991). [2] Robin C., Blanche F., Cauchois L., Cameron B., Couder M., Crouzet J. J. *Bacteriol.* 173:4893-4896(1991). [3] Crouzet J., Cameron B., Cauchois L., Rigault S., Rouyez M.-C., Blanche F., Thibaut D., Debussche L. J. *Bacteriol.* 172:5980-5990(1990). [4] Roth J.R., Lawrence J.G., Rubenfield M., Kieffer-Higgins S., Church G.M. J. *Bacteriol.* 175:3303-3316(1993). [5] Mattheakis L.C., Shen W.H., Collier R.J. *Mol. Cell. Biol.* 12:4026-4037(1992).

646. Tudor domain

Domain of unknown function present in several RNA-binding proteins. copies in the *Drosophila* Tudor protein. Slight ambiguities in the alignment. Number of members: 18

[1]Medline: 97200561 Tudor domains in proteins that interact with RNA. Ponting CP; Trends Biochem Sci 1997;22:51-52. [2]Medline: 97157029 The human EBNA-2 coactivator p100: multidomain organization and relationship to the staphylococcal nuclease fold and to the tudor protein involved in *Drosophila melanogaster* development. Callebaut I, Mornon JP; Biochem J 1997;321:125-132.

647. Terpene synthase family

It has been suggested that this gene family be designated

tps (for terpene synthase) [1]. It has been split into six

subgroups on the basis of phylogeny, called tpsa-tpsf.

tpsa includes vetispiradiene synthase Swiss:Q39979, 5-epi-aristolochene synthase, Swiss:Q40577 and (+)-delta-cadinene synthase Swiss:P93665.

tpsb includes (-)-limonene synthase, Swiss:Q40322.

tpsc includes kaurene synthase A, Swiss:O04408.

tpsd includes taxadiene synthase, Swiss:Q41594, pinene synthase, Swiss:O24475 and myrcene synthase, Swiss:O24474.

tpse includes kaurene synthase B.

tpsf includes linalool synthase.

Number of members: 51

[1]

Medline: 97413772

Monoterpene synthases from grand fir (*Abies grandis*). cDNA

isolation, characterization, and functional expression of

myrcene synthase, (-)-(4S)-limonene synthase, and

(-)-(1S,5S)-pinene synthase.

Bohlmann J, Steele CL, Croteau R;

J Biol Chem 1997;272:21784-21792.

648. ThiF family

This family contains a repeated domain in ubiquitin activating enzyme E1 and members of the bacterial

ThiF/MoeB/HesA family. Number of members: 87

649. Thioester dehydrase

- 5 Members of this family are involved in fatty acid biosynthesis.

Number of members: 19

[1]

Medline: 96398612

- 10 Structure of a dehydratase-isomerase from the bacterial pathway for biosynthesis of unsaturated fatty acids: two catalytic activities in one active site.

Leesong M, Henderson BS, Gillig JR, Schwab JM, Smith JL;

Structure 1996;4:253-264.

Database Reference: SCOP; 1mka; fa; [SCOP-USA][CATH-PDBSUM]

- 15 Database reference: PFAMB; PB058036;

650. Tub family signatures

- 20 The mouse tubby mutation is the cause of maturity-onset obesity, insulin resistance and sensory deficits. This mutation maps to a gene, tub [1,2], which codes for a protein that belongs to a family which currently consists of the following members: - Mammalian tub, an hydrophilic protein of about 500 residues, which could be involved in the hypothalamic regulation of body weight. - Human protein TULP1 [3] which may be involved in retinitis pigmentosa 14, a retinal degeneration disease. - Mouse protein p4-6 whose function is not
25 known. - Caenorhabditis elegans hypothetical protein F10B5.4. - Several fragmentary sequences from plants, Drosophila and human ESTs. While the N-terminal part of these protein is not conserved in length nor in the sequence, the C-terminal 250 residues are highly conserved. Therefore, two regions were selected in the C-terminal part as signature patterns. The second region is located at the C-terminal extremity and contains a penultimate cysteine
30 residue that could be critical to the normal functioning of these proteins.

Consensus pattern: F-[KHQ]-G-R-V-[ST]-x-A-S-V-K-N-F-Q

Consensus pattern: A-F-[AG]-I-[SAC]-[LIVM]-[ST]-S-F-x-[GST]-K-x-A-C-E

[1] Kleyn P.W., Fan W., Kovats S.G., Lee J.L., Pulido J.C., Wu Y., Berkemeier L.R.,

Misumi D.J., Holmgren L., Charlat O., Woolf E.A., Tayber O., Brody T., Shu P., Hawkins F.,

Kennedy B., Baldini L., Ebeling C., Alperin G.D., Deeds J., Lakey N.D., Culpepper J., Chen H., Gluecksmann-Kuis M.A., Carlson G.A., Duyk G.M., Moore K.J. *Cell* 85:281-290(1996).[2] Noben-Trauth K., Naggert J.K., North M.A., Nishina P.M. *Nature* 380:534-538(1996).[3] North M.A., Naggert J.K., Yan Y., Noben-Trauth K., Nishina P.M. *Proc. Natl. Acad. Sci. U.S.A.* 94:3128-3133(1997).

651. Eukaryotic DNA topoisomerase I active site

DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type I topoisomerases act by catalyzing the transient breakage of DNA, one strand at a time, and the subsequent rejoining of the strands. When a eukaryotic type I topoisomerase breaks a DNA backbone bond, it simultaneously forms a protein-DNA link where the hydroxyl group of a tyrosine residue is joined to a 3'-phosphate on DNA, at one end of the enzyme-severed DNA strand. In eukaryotes and pox virus topoisomerases I, there are a number of conserved residues in the region around the active site tyrosine.

Consensus pattern: [DEN]-x(6)-[GS]-[IT]-S-K-x(2)-Y-[LIVM]-x(3)-[LIVM] [Y is the active site tyrosine]

[1] Sternglanz R. *Curr. Opin. Cell Biol.* 1:533-535(1990).[2] Sharma A., Mondragon A. *Curr. Opin. Struct. Biol.* 5:39-47(1995).[3] Lynn R.M., Bjornsti M.-A., Caron P.R., Wang J.C. *Proc. Natl. Acad. Sci. U.S.A.* 86:3559-3563(1989).[4] Roca J. *Trends Biochem. Sci.* 20:156-160(1995).[E1]

652. Transaldolase signatures

Transaldolase (EC 2.2.1.2) catalyzes the reversible transfer of a three-carbonketol unit from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate to form erythrose 4-phosphate and fructose 6-phosphate. This enzyme, together with transketolase, provides a link between the glycolytic and pentose-phosphate pathways. Transaldolase is an enzyme of about 34 Kd whose sequence has been well conserved throughout evolution. A lysine has been implicated [1] in the catalytic mechanism of the enzyme; it acts as a nucleophilic group that attacks the carbonyl group of fructose-6-phosphate. Transaldolase is evolutionarily related [2] to a bacterial protein of about 20Kd (known as talC in *Escherichia coli*), whose exact function is not yet known. Two signature patterns have been developed for these proteins. The first,

located in the N-terminal section, contains a perfectly conserved pentapeptide; these cond, includes the active site lysine.

Consensus pattern: [DG]-[IVSA]-T-[ST]-N-P-[STA]-[LIVMF](2)

Consensus pattern: [LIVM]-x-[LIVM]-K-[LIVM]-[PAS]-x-[ST]-x-[DENQPAS]-G- [LIVM]-

5 x-[AGV]-x-[QEKIRST]-x-[LIVM] [K is the active site residue]

[1] Miosga T., Schaaff-Gerstenschlaeger I., Franken E., Zimmermann F.K. Yeast 9:1241-1249(1993).[2] Reizer J., Reizer A., Saier M.H. Jr. Microbiology 141:961-971(1995).

10 653. (Transpeptidase) Penicillin binding protein transpeptidase domain

The active site serine (residue 337 in Swiss:P14677) is conserved in all members of this family.

15 [1] Pares S, Mouz N, Petillot Y, Hakenbeck R, Dideberg O Nat Struct Biol 1996;3:284-289.

654. Trehalase signatures

Trehalase (EC 3.2.1.28) is the enzyme responsible for the degradation of the disaccharide

20 alpha, alpha-trehalose yielding two glucose subunits [1]. It is an enzyme found in a wide

variety of organisms and whose sequence has been highly conserved throughout evolution.

Two of the most highly conserved regions have been selected as signature patterns. The first pattern is located in the central section, the second one is in the C-terminal region.

Consensus pattern: P-G-G-R-F-x-E-x-Y-x-W-D-x-Y

25 Consensus pattern: Q-W-D-x-P-x-[GA]-W-[PAS]-P

[1] Kopp M., Mueller H., Holzer H. J. Biol. Chem. 268:4766-4774(1993).[2] Henrissat B., Bairoch A. Biochem. J. 293:781-788(1993).[E1]

30 655. Trehalose-6-phosphate synthase domain

OtsA (Trehalose-6-phosphate synthase) is homologous to regions

in the subunits of yeast trehalose-6-phosphate synthase/phosphate complex, [1].

[1] Kaasen I, McDougall J, Strom AR; Gene 1994;145:9-15.

656. Tropomyosins signature

Tropomyosins [1,2] are family of closely related proteins present in muscle and non-muscle cells. In striated muscle, tropomyosin mediate the interactions between the troponin complex and actin so as to regulate muscle contraction. The role of tropomyosin in smooth muscle and non-muscle tissues is not clear. Tropomyosin is an alpha-helical protein that forms a coiled-coil dimer. Muscle isoforms of tropomyosin are characterized by having 284 amino acid residues and a highly conserved N-terminal region, whereas non-muscle forms are generally smaller and are heterogeneous in their N-terminal region. The signature pattern for tropomyosins is based on a very conserved region in the C-terminal section of tropomyosins and which is present in both muscle and non-muscle forms.

Consensus pattern: L-K-E-A-E-x-R-A-E

[1] Smilie L.B. Trends Biochem. Sci. 4:151-155(1979).[2] McLeod A.R. BioEssays 6:208-212(1986).

657. Troponin

Troponin (Tn) contains three subunits, Ca²⁺ binding (TnC), inhibitory (TnI), and tropomyosin binding (TnT). this Pfam contains members of the TnT subunit.

Troponin is a complex of three proteins, Ca²⁺ binding (TnC), inhibitory (TnI), and tropomyosin binding (TnT).

The troponin complex regulates Ca⁺⁺ induced muscle contraction.

This family includes troponin T and troponin I. Troponin I

binds to actin and troponin T binds to tropomyosin.

Number of members: 81 [1]

Medline: 87144593

Structure of co-crystals of tropomyosin and troponin.

White SP, Cohen C, Phillips GN Jr;

Nature 1987;325:826-828. [2]

Medline: 95155315

A direct regulatory role for troponin T and a dual role for troponin C in the Ca²⁺ regulation of muscle contraction.

Potter JD, Sheng Z, Pan BS, Zhao J;

J Biol Chem 1995;270:2557-2562.

[3]Medline: 95324796

The troponin complex and regulation of muscle contraction.

Farah CS, Reinach FC;

5 FASEB J 1995;9:755-767.

658. (Tryp mucin) Mucin-like glycoprotein

- 10 This family of trypanosomal proteins resemble vertebrate mucins. The protein consists of three regions. The N and C terminii are conserved between all members of the family, whereas the central region is not well conserved and contains a large number of threonine residues which can be glycosylated [1].
- Indirect evidence suggested that these genes might encode the core protein of parasite
- 15 mucins, glycoproteins that were proposed to be involved in the interaction with, and invasion of, mammalian host cells.
- [1] Di Noia JM, Sanchez DO, Frasch AC; J Biol Chem 1995;270:24146-24149.
- [2] Di Noia JM, D'Orso I, Aslund L, Sanchez DO, Frasch AC; J Biol Chem 1998;273:10843-20 10850.

659. Aminoacyl-transfer RNA synthetases class-I signature (tRNA synt 1)

- Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino
- 25 acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely
- 30 diverse in terms of subunit size and of quaternary structure. A few years ago it was found [2] that several aminoacyl-tRNA synthetases share a region of similarity in their N-terminal section, in particular the consensus tetrapeptide His-Ile-Gly-His ('HIGH') is very well conserved. The 'HIGH' region has been shown [3] to be part of the adenylate binding site. The 'HIGH' signature has been found in the aminoacyl-tRNA synthetases specific for

arginine, cysteine, glutamic acid, glutamine, isoleucine, leucine, methionine, tyrosine, tryptophan, and valine. These aminoacyl-tRNA synthetases are referred to as class-I synthetases [4,5,6] and seem to share the same tertiary structure based on a Rossmann fold. Consensus pattern: P-x(0,2)-[GSTAN]-[DENQGAPK]-x-[LIVMFPP]-[HT]-[LIVMYAC]-G-

[HNTG]-[LIVMFYSTAGPC]

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Webster T., Tsai H., Kula M., Mackie G.A., Schimmel P. Science 226:1315-1317(1984).[3] Brick P., Bhat T.N., Blow D.M. J. Mol. Biol. 208:83-98(1988).[4] Delarue M., Moras D. BioEssays 15:675-687(1993).[5] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[6] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).

660. Aminoacyl-transfer RNA synthetases class-I signature (tRNA synt 1b)

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. A few years ago it was found [2] that several aminoacyl-tRNA synthetases share a region of similarity in their N-terminal section, in particular the consensus tetrapeptide His-Ile-Gly-His ('HIGH') is very well conserved. The 'HIGH' region has been shown [3] to be part of the adenylate binding site.

The 'HIGH' signature has been found in the aminoacyl-tRNA synthetases specific for arginine, cysteine, glutamic acid, glutamine, isoleucine, leucine, methionine, tyrosine, tryptophan, and valine. These aminoacyl-tRNA synthetases are referred to as class-I synthetases [4,5,6] and seem to share the same tertiary structure based on a Rossmann fold. Consensus pattern: P-x(0,2)-[GSTAN]-[DENQGAPK]-x-[LIVMFPP]-[HT]-[LIVMYAC]-G-[HNTG]-[LIVMFYSTAGPC]

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Webster T., Tsai H., Kula M., Mackie G.A., Schimmel P. Science 226:1315-1317(1984).[3] Brick P., Bhat T.N., Blow D.M. J. Mol. Biol. 208:83-98(1988).[4] Delarue M., Moras D. BioEssays 15:675-687(1993).[5] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[6] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).

661. (tRNA-synt 1C) tRNA synthetases class I (E and Q)

- 5 Other tRNA synthetase sub-families are too dissimilar to be included.

This family includes only glutamyl and glutaminyl tRNA synthetases.

In some organisms, a single glutamyl-tRNA synthetase aminoacylates both tRNA(Glu) and tRNA(Gln).

- 10 [1] Rath VL, Silvian LF, Beijer B, Sproat BS, Steitz TA; Structure 1998;6:439-449.

662. (tRNA-synt 1d) tRNA synthetases class I (R)

- 15 Other tRNA synthetase sub-families are too dissimilar to be included.

This family includes only arginyl tRNA synthetase.

663. Aminoacyl-transfer RNA synthetases class-II signatures (tRNA synt 2)

- 20 Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a
25 mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. The synthetases specific for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is
30 different to the Rossmann fold observed for the class I synthetases [7]. Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions are present [2,5,8]. Signature patterns have been derived from two of these regions.
Consensus pattern: [FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE]

Consensus pattern: [GSTALVF]-{DENQHRKP}-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-[LIVMSTAG]-[LIVMFY]

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Delarue M., Moras D. BioEssays 15:675-687(1993).[3] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[4] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991). [5] Cusack S., Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991).[6] Cusack S. Biochimie 75:1077-1081(1993).[7] Cusack S., Berthet-Colominas C., Haertlein M., Nassar N., Leberman R. Nature 347:249-255(1990).[8] Leveque F., Plateau P., Dessen P., Blanquet S. Nucleic Acids Res. 18:305-312(1990).

664. Aminoacyl-transfer RNA synthetases class-I signature (tRNA synt 1e)
Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. A few years ago it was found [2] that several aminoacyl-tRNA synthetases share a region of similarity in their N-terminal section, in particular the consensus tetrapeptide His-Ile-Gly-His ('HIGH') is very well conserved. The 'HIGH' region has been shown [3] to be part of the adenylate binding site. The 'HIGH' signature has been found in the aminoacyl-tRNA synthetases specific for arginine, cysteine, glutamic acid, glutamine, isoleucine, leucine, methionine, tyrosine, tryptophan, and valine. These aminoacyl-tRNA synthetases are referred to as class-I synthetases [4,5,6] and seem to share the same tertiary structure based on a Rossmann fold.
Consensus pattern: P-x(0,2)-[GSTAN]-[DENQGAPK]-x-[LIVMFP]-[HT]-[LIVMYAC]-G-[HNTG]-[LIVMFYSTAGPC]
[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Webster T., Tsai H., Kula M., Mackie G.A., Schimmel P. Science 226:1315-1317(1984).[3] Brick P., Bhat T.N., Blow D.M. J. Mol. Biol. 208:83-98(1988).[4] Delarue M., Moras D. BioEssays 15:675-687(1993).[5] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[6] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).

665. Aminoacyl-transfer RNA synthetases class-II signatures (tRNA synt 2b)

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In

5 prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. The synthetases specific for
10 alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is different to the Rossmann fold observed for the class I synthetases [7]. Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions
15 are present [2,5,8]. Signature patterns have been derived from two of these regions.

Consensus pattern: [FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE]

Consensus pattern: [GSTALVF]-{DENQHRKP}-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-[LIVMSTAG]-[LIVMFY]

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987). [2] Delarue M., Moras D.

20 BioEssays 15:675-687(1993). [3] Schimmel P. Trends Biochem. Sci. 16:1-3(1991). [4] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991). [5] Cusack S., Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991). [6] Cusack S. Biochimie 75:1077-1081(1993). [7] Cusack S., Berthet-Colominas C., Haertlein M., Nassar N., Leberman R. Nature 347:249-255(1990). [8] Leveque F., Plateau P., Dessen P., Blanquet
25 S. Nucleic Acids Res. 18:305-312(1990).

666. Thaumatin family signature

Thaumatococcus danianii, an African brush. The protein is made of about
30 a molar basis) from Thaumatooccus daniellii, an African brush. The protein is made of about 200 residues and contains 8 disulfide bonds. A number of proteins have been found to be related to thaumatins. These proteins are listed below (references are only provided for recently determined sequences). - A maize alpha-amylase/trypsin inhibitor. - Two tobacco pathogenesis-related proteins: PR-R major and minor forms, which are induced after

infection with viruses. - Salt-induced protein NP24 from tomato. - Osmotin, a salt-induced protein from tobacco. - Osmotin-like proteins OSML13, OSML15 and OSML81 from potato [2]. - P21, a leaf protein from soybean. - PWIR2, a leaf protein from wheat. - Zeamatin, a maize antifungal protein [3]. The exact biological function of all these proteins is not yet known. A conserved region that includes three cysteine residues known (in thaumatin) to be involved in disulfide bonds has been selected as a signature pattern.

```
+-----+ | +-----+ || ***** |
||
```

```
xxCXXXXXXXXXXXXCxCxCxCXXXXXXXXXXXXCxCxCxxxCxCxCxxxCxxxCCCCCxCxxxCCCCC
```

```
10 xxxCx ||||| ||| +--+ ++ | +--+ +--+ | +-----+ 'C': conserved cysteine
involved in a disulfide bond. '*': position of the pattern.
```

Consensus pattern: G-x-[GF]-x-C-x-T-[GA]-D-C-x(1,2)-G-x(2,3)-C

[1] Edens L., Heslinga L., Klok R., Ledebore A.M., Maat J., Toonen M.Y., Visser C., Verrips C.T. Gene 18:1-12(1982). [2] Zhu B., Chen T.H.H., Li P.H. Plant Physiol. 108:929-937(1995). [3] Malehorn D.E., Borgmeyer J.R., Smith C.E., Shah D.M.; Plant Physiol. 106:1471-1481(1994).

667. Thiolases signatures

Two different types of thiolase [1,2,3] are found both in eukaryotes and in prokaryotes: acetoacetyl-CoA thiolase (EC 2.3.1.9) and 3-ketoacyl-CoA thiolase (EC 2.3.1.16). 3-ketoacyl-CoA thiolase (also called thiolase I) has a broad chain-length specificity for its substrates and is involved in degradative pathways such as fatty acid beta-oxidation. Acetoacetyl-CoA thiolase (also called thiolase II) is specific for the thiolysis of acetoacetyl-CoA and involved in biosynthetic pathways such as poly beta-hydroxybutyrate synthesis or steroid biogenesis. In eukaryotes, there are two forms of 3-ketoacyl-CoA thiolase: one located in the mitochondrion and the other in peroxisomes. There are two conserved cysteine residues important for thiolase activity. The first located in the N-terminal section of the enzymes is involved in the formation of an acyl-enzyme intermediate; the second located at the C-terminal extremity is the active site base involved in deprotonation in the condensation reaction. Mammalian nonspecific lipid-transfer protein (nsL-TP) (also known as sterol carrier protein 2) is a protein which seems to exist in two different forms: a 14 Kd protein (SCP-2) and a larger 58 Kd protein (SCP-x). The former is found in the cytoplasm or the mitochondria and is involved in lipid transport; the latter is found in peroxisomes. The C-terminal part of SCP-x is identical to

SCP-2 while the N-terminal portion is evolutionary related to thiolases[4]. Three signature patterns have been developed for this family of proteins, two of which are based on the regions around the biologically important cysteines. The third is based on a highly conserved region in the C-terminal part of these proteins.

- 5 Consensus pattern: [LIVM]-[NST]-x(2)-C-[SAGLI]-[ST]-[SAG]-[LIVMFYNS]-x- [STAG]-[LIVM]-x(6)-[LIVM] [C is involved in formation of acyl-enzyme intermediate]
 Consensus pattern: N-x(2)-G-G-x-[LIVM]-[SA]-x-G-H-P-x-[GA]-x-[ST]-G
 Consensus pattern: [AG]-[LIVMA]-[STAGCLIVM]-[STAG]-[LIVMA]-C-x-[AG]-x-[AG]-x-[AG]-x-[SAG] [C is the active site residue]

- 10 [1] Peoples O.P., Sinskey A.J. J. Biol. Chem. 264:15293-15297(1989).[2] Yang S.-Y., Yang X.-Y.H., Healy-Louie G., Schulz H., Elzinga M. J. Biol. Chem. 265:10424-10429(1990).[3] Igual J.C., Gonzalez-Bosch C., Dopazo J., Perez-Ortin J.E. J. Mol. Evol. 35:147-155(1992).[4] Baker M.E., Billheimer J.T., Strauss J.F. III DNA Cell Biol. 10:695-698(1991).

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668. Thioredoxin family active site

- Thioredoxins [1 to 4] are small proteins of approximately one hundred amino-acid residues which participate in various redox reactions via the reversible oxidation of an active center disulfide bond. They exist in either a reduced form or an oxidized form where the two cysteine residues are linked in an intramolecular disulfide bond. Thioredoxin is present in prokaryotes and eukaryotes and the sequence around the redox-active disulfide bond is well conserved. Bacteriophage T4 also encodes for a thioredoxin but its primary structure is not homologous to bacterial, plant and vertebrate thioredoxins. A number of eukaryotic proteins contain domains evolutionary related to thioredoxin, all of them seem to be protein disulphide isomerases (PDI). PDI(EC 5.3.4.1) [5,6,7] is an endoplasmic reticulum enzyme that catalyzes the rearrangement of disulfide bonds in various proteins. The various forms of PDI which are currently known are: - PDI major isozyme; a multifunctional protein that also function as the beta subunit of prollyl 4-hydroxylase (EC 1.14.11.2), as a component of oligosaccharyl transferase (EC 2.4.1.119), as thyroxine deiodinase (EC 3.8. 1.4), as
- 25 glutathione-insulin transhydrogenase (EC 1.8.4.2) and as a thyroid hormone-binding protein !
- 30 - ERp60 (ER-60; 58 Kd microsomal protein). ERp60 was originally thought to be a phosphoinositide-specific phospholipase C isozyme and later to be a protease. - ERp72. - P5.AII PDI contains two or three (ERp72) copies of the thioredoxin domain. Bacterial proteins that act as thiol:disulfide interchange proteins that allows disulfide bond formation in

some periplasmic proteins also contain a thioredoxin domain. These proteins are: -

Escherichia coli dsbA (or prfA) and its orthologs in Vibrio cholerae (tcpG) and Haemophilus influenzae (por). - Escherichia coli dsbC (or xpRA) and its orthologs in Erwinia chrysanthemi and Haemophilus influenzae. - Escherichia coli dsbD (or dipZ) and its Haemophilus

- 5 influenzae ortholog. - Escherichia coli dsbE (or ccmG) and orthologs in Haemophilus influenzae, Rhodobacter capsulatus (helX), Rhizobiaceae (cycY and tlpA).
Consensus pattern: [LIVMF]-[LIVMSTA]-x-[LIVMFYC]-[FYWSTHE]-x(2)-[FYWGTN]-C-[GATPLVE]-[PHYWSTA]-C-x(6)-[LIVMFYWT] [The two C's form the redox-active bond]

- 10 [1] Holmgren A. Annu. Rev. Biochem. 54:237-271(1985).[2] Gleason F.K., Holmgren A. FEMS Microbiol. Rev. 54:271-297(1988).[3] Holmgren A. J. Biol. Chem. 264:13963-13966(1989).[4] Eklund H., Gleason F.K., Holmgren A. Proteins 11:13-28(1991).[5] Freedman R.B., Hawkins H.C., Murrant S.J., Reid L. Biochem. Soc. Trans. 16:96-99(1988).[6] Kivirikko K.I., Myllylä R., Pihlajaniemi T. FASEB J. 3:1609-1617(1989).[7] Freedman R.B., Hirst T.R., Tuite M.F. Trends Biochem. Sci. 19:331-336(1994).

669. (Transcript fac2) Transcription factor TFIIB repeat signature

- In eukaryotes the initiation of transcription of protein encoding genes by polymerase II is modulated by general and specific transcription factors. The general transcription factors operate through common promoters elements (such as the TATA box). At least seven different proteins associates to form the general transcription factors: TFIIA, -IIB, -IID, -IIE, -IIF, -IIG, and -IIH[1]. Transcription factor IIB (TFIIB) plays a central role in the transcription of class II genes, it associates with a complex of TFIID-IIA bound to DNA (DA complex) to form a ternary complex TFIID-IIA-IBB (DAB complex) which is then recognized by RNA polymerase II [2,3]. TFIIB is a protein of about 315 to 340 amino acid residues which contains, in its C-terminal part an imperfect repeat of a domain of about 75 residues. This repeat could contribute an element of symmetry to the folded protein. The following proteins have been shown to be evolutionary related to TFIIB: - An archaeobacterial
- 20 TFIIB homolog. In Pyrococcus woesei a previously undetected open reading frame has been shown [4] to be highly related to TFIIB. - Fungal transcription factor IIIB 70 Kd subunit (gene PCF4/TDS4/BRF1) [5]. This protein is a general activator of RNA polymerase III transcription and plays a role analogous to that of TFIIB in pol III transcription. The central
- 30

section of the repeated domain, which is the most conserved part of that domain has been selected as a signature pattern.

Consensus pattern: G-[KR]-x(3)-[STAGN]-x-[LIVMYA]-[GSTA](2)-[CSAV]-[LIVM]-[LIVMFY]-[LIVMA]-[GSA]-[STAC]

- 5 [1] Weinmann R. *Gene Expr.* 2:81-91(1992).[2] Hawley D. *Trends Biochem. Sci.* 16:317-318(1991).[3] Ha I., Lane W.S., Reinberg D. *Nature* 352:689-695(1991).[4] Ouzounis C., Sander C. *Cell* 71:189-190(1992).[5] Khoo B., Brophy B., Jackson S.P. *Genes Dev.* 8:2879-2890(1994).

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670. (transcript fact) MADS-box domain signature and profile

- A number of transcription factors contain a conserved domain of 56 amino-acid residues, sometimes known as the MADS-box domain [E1]. They are listed below: - Serum response factor (SRF) [1], a mammalian transcription factor that binds to the Serum Response Element (SRE). This is a short sequence of dyad symmetry located 300 bp to the 5' end of the transcription initiation site of genes such as c-fos. - Mammalian myocyte-specific enhancer factors 2A to 2D (MEF2A to MEF2D). These proteins are transcription factor which binds specifically to the MEF2 element present in the regulatory regions of many muscle-specific genes. - Drosophila myocyte-specific enhancer factor 2 (MEF2). - Yeast GRM/PRTF protein (gene MCM1) [2], a transcriptional regulator of mating-type-specific genes. - Yeast arginine metabolism regulation protein I (gene ARGRI or ARG80). - Yeast transcription factor RLM1. - Yeast transcription factor SMP1. - Arabidopsis thaliana agamous protein (AG) [3], a probable transcription factor involved in regulating genes that determines stamen and carpel development in wild-type flowers. Mutations in the AG gene result in the replacement of the stamens by petals and the carpels by a new flower. - Arabidopsis thaliana homeotic proteins Apetala1 (AP1), Apetala3 (AP3) and Pistillata (PI) which act locally to specify the identity of the floral meristem and to determine sepal and petal development [4]. - Antirrhinum majus and tobacco homeotic protein deficiens (DEFA) and globosa (GLO) [5]. Both proteins are transcription factors involved in the genetic control of flower development. Mutations in DEFA or GLO cause the transformation of petals into sepals and of stamens into carpels. - Arabidopsis thaliana putative transcription factors AGL1 to AGL6 [6]. - Antirrhinum majus morphogenetic protein DEF H33 (squamosa). In SRF, the conserved domain has been shown [1] to be involved in DNA-binding and dimerization. A pattern that spans the complete length of the domain has been derived. The profile also spans the length of the MADS-box.

Consensus pattern: R-x-[RK]-x(5)-I-x-[DNGSK]-x(3)-[KR]-x(2)-T-[FY]-x-[RK](3)-x(2)-[LIVM]-x-K(2)-A-x-E-[LIVM]-[STA]-x-L-x(4)-[LIVM]-x-[LIVM](3)-x(6)-[LIVMF]-x(2)-[FY]

[1] Norman C., Runswick M., Pollock R., Treisman R. *Cell* 55:989-1003(1988).[2]

- 5 Passmore S., Maine G.T., Elble R., Christ C., Tye B.-K. *J. Mol. Biol.* 204:593-606(1988).[3] Yanofsky M., Ma H., Bowman J., Drews G., Feldmann K.A., Meyerowitz E.M. *Nature* 346:35-39(1990).[4] Goto K., Meyerowitz E.M. *Genes Dev.* 8:1548-1560(1994).[5] Troebner W., Ramirez L., Motte P., Hue I., Huijser P., Loennig W.-E., Saedler H., Sommer H., Schwartz-Sommer Z. *EMBO J.* 11:4693-4704(1992).[6] Ma H., Yanofsky M.F.,
10 Meyerowitz E.M. *Genes Dev.* 5:484-495(1991).[E1]

671. Transketolase signatures

- 15 Transketolase (EC 2.2.1.1) (TK) catalyzes the reversible transfer of a two-carbon ketol unit from xylulose 5-phosphate to an aldose receptor, such as ribose 5-phosphate, to form sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate. This enzyme, together with transaldolase, provides a link between the glycolytic and pentose-phosphate pathways. TK requires thiamin pyrophosphate as a cofactor. In most sources where TK has been purified, it is a homodimer of approximately 70 Kd subunits. TK sequences from a variety of eukaryotic
20 and prokaryotic sources [1,2] show that the enzyme has been evolutionarily conserved. In the peroxisomes of methylotrophic yeast *Hansenula polymorpha*, there is a highly related enzyme, dihydroxy-acetone synthase (DHAS) (EC 2.2.1.3) (also known as formaldehyde transketolase), which exhibits a very unusual specificity by including formaldehyde amongst its substrates. 1-deoxyxylulose-5-phosphate synthase (DXP synthase) [3] is an enzyme so far
25 found in bacteria (gene *dxs*) and plants (gene *CLA1*) which catalyzes the thiamin pyrophosphate-dependent acyloin condensation reaction between carbon atoms 2 and 3 of pyruvate and glyceraldehyde 3-phosphate to yield 1-deoxy-D- xylulose-5-phosphate (*dxp*), a precursor in the biosynthetic pathway to isoprenoids, thiamin (vitamin B1), and pyridoxol (vitamin B6). DXP synthase is evolutionary related to TK. Two regions of TK have been
30 selected as signature patterns. The first, located in the N-terminal section, contains a histidine residue which appears to function in proton transfer during catalysis [4]. The second, located in the central section, contains conserved acidic residues that are part of the active cleft and may participate in substrate-binding [4].

Consensus pattern: R-x(3)-[LIVMTA]-[DENQSTHKF]-x(5,6)-[GSN]-G-H-[PLIVMF]-
[GSTA]-x(2)-[LIMC]-[GS]

Consensus pattern: G-[DEQGSA]-[DN]-G-[PAEQ]-[ST]-[HQ]-x-[PAGM]-[LIVMYAC]-
[DEFYW]-x(2)-[STAP]-x(2)-[RGA]

- 5 [1] Abedinia M., Layfield R., Jones S.M., Nixon P.F., Mattick J.S. *Biochem. Biophys. Res. Commun.* 183:1159-1166(1992).[2] Fletcher T.S., Kwee I.L., Nakada T., Largman C., Martin B.M. *Biochemistry* 31:1892-1896(1992).[3] Sprenger G.A., Schorken U., Wiegert T., Grolle S., De Graaf A.A., Taylor S.V., Begley T.P., Bringer-Meyer S., Sahm H. Proc. Natl. Acad. Sci. U.S.A. 94:12857-12862(1997).[4] Lindqvist Y., Schneider G., Ermler U., Sundstroem M. *EMBO J.* 11:2373-2379(1992).

672. Transmembrane 4 family signature

- 15 Recently a number of eukaryotic cell surface antigens have been found to be evolutionary related [1,2,3]. The proteins known to belong to this family are listed below: - Mammalian antigen CD9 (MIC3); A protein involved in platelet activation and aggregation. - Mammalian leukocyte antigen CD37, expressed on B lymphocytes. - Mammalian leukocyte antigen CD53 (OX-44), which may be involved in growth regulation in hematopoietic cells. - Mammalian lysosomal membrane protein CD63 (melanoma-associated antigen ME491; antigen AD1).
- 20 Mammalian antigen CD81 (cell surface protein TAPA-1), which may play an important role in the regulation of lymphoma cell growth. - Mammalian antigen CD82 (protein R2; antigen C33; Kangai 1 (KAI1)), which associates with CD4 or CD8 and delivers costimulatory signals for the TCR/CD3 pathway. - Mammalian antigen CD151 (SFA-1; platelet-endothelial tetraspan antigen 3 (PETA-3)). - Mammalian cell surface glycoprotein A15 (TALLA-1;
- 25 MXS1). - Mammalian novel antigen 2 (NAG-2). - Human tumor-associated antigen CO-029. - *Schistosoma mansoni* and *japonicum* 23 Kd surface antigen (SM23 / SJ23). These proteins share the following characteristics: they all seem to be type III membrane proteins (type III proteins are integral membrane proteins that contain a N-terminal membrane-anchoring domain which is not cleaved during biosynthesis and which functions both as a translocation
- 30 signal and as a membrane anchor); they also contain three additional transmembrane regions, at least seven conserved cysteines residues, and are of approximately the same size (218 to 284 residues). These proteins are collectively know as the 'transmembrane 4 super family' (TM4) because they span the plasma membrane four times. A schematic diagram of the domain structure of these proteins is shown below. +-----+-----+-----+-----+-----

-----+-----+ || TMa | Extra | TM2| Cyt | TM3 | Extracellular | TM4 | Cyt| +-----
+-----+---C---C-----CC-----C---C-----+-----+ *****Cyt : cytoplasmic
domain. TMa : transmembrane anchor.TM2 to TM4: transmembrane regions 2 to 4.'C' :
conserved cysteine. '*' : position of the pattern.

- 5 A conserved region that includes two cysteines and seems to be located in a short
cytoplasmic loop between two transmembrane domains has been selected as a signature for
these proteins.

Consensus pattern: G-x(3)-[LIVMF]-x(2)-[GSA]-[LIVMF](2)-G-C-x-[GA]-[STA]- x(2)-
[EG]-x(2)-[CWN]-[LIVM](2)

- 10 [1] Levy S., Nguyen V.Q., Andria M.L., Takahashi S. J. Biol. Chem. 266:14597-
14602(1991).[2] Tomlinson M.G., Williams A.F., Wright M.D. Eur. J. Immunol. 23:136-
40(1993).[3] Barclay A.N., Birkeland M.L., Brown M.H., Beyers A.D., Davis S.J., Somoza
C., Williams A.F. The leucocyte antigen factbooks. Academic Press, London / San Diego,
(1993).

673. Tryptophan synthase alpha chain signature

Tryptophan synthase catalyzes the last step in the biosynthesis of tryptophan: the conversion
of indoleglycerol phosphate and serine, totryptophan and glyceraldehyde 3-phosphate [1,2]. It
has two functional domains: one for the aldol cleavage of indoleglycerol phosphate to indole
andglyceraldehyde 3-phosphate and the other for the synthesis of tryptophan fromindole and
serine. In bacteria and plants [3], each domain is found on a separate subunit (alpha and beta
chains), while in fungi the two domains are fused together on a single multifunctional protein.
A conserved region that contains three conserved acidic residues has been selected as a
signature pattern for the alpha chain. The first and the third acidic residues are believed to
serve as proton donors/acceptors in the enzyme's catalytic mechanism.

Consensus pattern: [LIVM]-E-[LIVM]-G-x(2)-[FYC]-[ST]-[DE]-[PA]-[LIVMY]- [AGLI]-
[DE]-G

- [1] Crawford I.P. Annu. Rev. Microbiol. 43:567-600(1989).[2] Hyde C.C., Miles E.W.
30 Bio/Technology 8:27-32(1990).[3] Berlyn M.B., Last R.L., Fink G.R. Proc. Natl. Acad. Sci.
U.S.A. 86:4604-4608(1989).

674. Tryptophan synthase beta chain pyridoxal-phosphate attachment site

Tryptophan synthase catalyzes the last step in the biosynthesis of tryptophan: the conversion of indoleglycerol phosphate and serine, to tryptophan and glyceraldehyde 3-phosphate [1,2]. It has two functional domains: one for the aldol cleavage of indoleglycerol phosphate to indole and glyceraldehyde 3-phosphate and the other for the synthesis of tryptophan from indole and serine. In bacteria and plants [3], each domain is found on a separate subunit (alpha and beta chains), while in fungi the two domains are fused together on a single multifunctional protein. The beta chain of the enzyme requires pyridoxal-phosphate as a cofactor. The pyridoxal-phosphate group is attached to a lysine residue. The region around this lysine residue also contains two histidine residues which are part of the pyridoxal-phosphate binding site. The signature pattern for the tryptophan synthase beta chain is derived from that conserved region. -Consensus pattern: [LIVM]-x-H-x-G-[STA]-H-K-x-N [K is the pyridoxal-P attachment site] [1] Crawford I.P. Annu. Rev. Microbiol. 43:567-600(1989). [2] Hyde C.C., Miles E.W. Bio/Technology 8:27-32(1990). [3] Berlyn M.B., Last R.L., Fink G.R. Proc. Natl. Acad. Sci. U.S.A. 86:4604-4608(1989).

675. Serine proteases, trypsin family, active sites

The catalytic activity of the serine proteases from the trypsin family is provided by a charge relay system involving an aspartic acid residue hydrogen-bonded to a histidine, which itself is hydrogen-bonded to a serine. The sequences in the vicinity of the active site serine and histidine residues are well conserved in this family of proteases [1]. A partial list of proteases known to belong to the trypsin family is shown below. - Acrosin. - Blood coagulation factors VII, IX, X, XI and XII, thrombin, plasminogen, and protein C. - Cathepsin G. - Chymotrypsins. - Complement components C1r, C1s, C2, and complement factors B, D and I. - Complement-activating component of RA-reactive factor. - Cytotoxic cell proteases (granzymes A to H). - Duodenase I. - Elastases 1, 2, 3A, 3B (protease E), leukocyte (medullasin). - Enterokinase (EC 3.4.21.9) (enteropeptidase). - Hepatocyte growth factor activator. - Hepsin. - Glandular (tissue) kallikreins (including EGF-binding protein types A, B, and C, NGF-gamma chain, gamma-renin, prostate specific antigen (PSA) and tonin). - Plasma kallikrein. - Mast cell proteases (MCP) 1 (chymase) to 8. - Myeloblastin (proteinase 3) (Wegener's autoantigen). - Plasminogen activators (urokinase-type, and tissue-type). - Trypsins I, II, III, and IV. - Trypsases. - Snake venom proteases such as ancrod, batroxobin, cerastobin, flavoxobin, and protein C activator. - Collagenase from common cattle grub and collagenolytic protease from Atlantic sand fiddler crab. - Apolipoprotein(a). - Blood fluke

carcerial protease. - *Drosophila* trypsin like proteases: alpha, easter, snake-locus. - *Drosophila* protease stubble (gene sb). - Major mite fecal allergen Der p III. All the above proteins belong to family S1 in the classification of peptidases[2,E1] and originate from eukaryotic species. It should be noted that bacterial proteases that belong to family S2A are similar enough in the regions of the active site residues that they can be picked up by the same patterns. These proteases are listed below. - *Achromobacter* lyticus protease I. - *Lysobacter* alpha-lytic protease. - *Streptogrisin* A and B (*Streptomyces* proteases A and B). - *Streptomyces* griseus glutamyl endopeptidase II. - *Streptomyces* fradiae proteases 1 and 2. Consensus pattern: [LIVM]-[ST]-A-[STAG]-H-C [H is the active site residue]

10 Consensus pattern: [DNSTAGC]-[GSTAPIMVQH]-x(2)-G-[DE]-S-G-[GS]-[SAPHV]-[LIVMFYWH]-[LIVMFYSTANQH] [S is the active site residue]

[1] Brenner S. Nature 334:528-530(1988).[2] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).[E1]

15 676. (tsp) Thrombospondin type 1 domain

[1] Bork P; FEBS lett 1993;327:125-130.

20 677. Tubulin subunits alpha, beta, and gamma signature

Tubulins [1,2], the major constituent of microtubules are dimeric proteins which consist of two closely related subunits (alpha and beta). Tubulin binds two molecules of GTP at two different sites (N and E). At the E (Exchangeable) site, GTP is hydrolyzed during incorporation into the microtubule. Near the E site is an invariant region rich in glycines which is found in both chains and which is now [3] said to control the access of the nucleotide to its binding site. A signature pattern was developed from this region. With the exception of the simple eukaryotes, most species express a variety of closely related alpha and beta isotypes. In most species there is a third member of the tubulin family: gamma tubulin.

25 Gamma tubulin is found at microtubule organizing centers (MTOC) such as the spindle poles or the centrosome, suggesting that it is involved in the minus-end nucleation of microtubule assembly [4].

30 Consensus pattern: [SAG]-G-G-T-G-[SA]-G

[1] Cleveland D.W., Sullivan K.F. *Annu. Rev. Biochem.* 54:331-365(1985), [2] Joshi H.C., Cleveland D.W. *Cell Motil. Cytoskeleton* 16:159-163(1990), [3] Hesse J., Thierauf M., Ponstingl H. *J. Biol. Chem.* 262:15472-15475(1987), [4] Joshi H.C. *BioEssays* 15:637-643(1993).

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Tubulin-beta mRNA autoregulation signal

The stability of beta-tubulin mRNAs are autoregulated by their own translation product [1]. Unpolymerized tubulin subunits bind directly (or activate a factor(s) which binds co-translationally) to the nascent N-terminus of beta-tubulin. This binding is transduced through the adjacent ribosomes to activate an RNase that degrades the polysome-bound mRNA. The recognition element has been shown to be the first four amino acids of beta-tubulin: Met-Arg-Glu-Ile. Mutations to this sequence abolish the autoregulation effect (except for the replacement of Glu by Asp); transposition of this sequence to an internal region of a polypeptide also suppresses the autoregulatory effect.

10

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Consensus pattern: <M-R-[DE]-[IL]

[1] Cleveland D.W. *Trends Biochem. Sci.* 13:339-343(1988).

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678. (tRNA-synt 2c) Aminoacyl-transfer RNA synthetases class-II signatures. Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. The synthetases specific for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is different to the Rossmann fold observed for the class I synthetases [7]. Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions are present [2,5,8]. Signature patterns have been derived from two of these regions.

Consensus pattern: [FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE]-

Consensus pattern: [GSTALVF]-{DENQHRKP}-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-
[LIVMSTAG]-[LIVMFY]-

- [1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987),[2] Delarue M., Moras D.
5 BioEssays 15:675-687(1993),[3] Schimmel P. Trends Biochem. Sci. 16:1-3(1991),[4] Nagel
G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991). [5] Cusack S.,
Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991),[6] Cusack S.
Biochimie 75:1077-1081(1993),[7] Cusack S., Berthet-Colominas C., Haertlein M., Nassar
N., Leberman R. Nature 347:249-255(1990),[8] Leveque F., Plateau P., Dessen P., Blanquet
10 S. Nucleic Acids Res. 18:305-312(1990).

679. UBA-domain

- The UBA-domain (ubiquitin associated domain) is a novel sequence motif found in
15 several proteins having connections to ubiquitin and the ubiquitination pathway. The
structure of the UBA domain consists of a compact three helix bundle [1]. Number of
members: 84

- [1] Structure of a human DNA repair protein UBA domain that interacts with HIV-1
Vpr. Dieckmann T, Withers-Ward ES, Jarosinski MA, Liu CF, Chen IS, Feigon J; Nat Struct
20 Biol 1998;5:1042-1047.

680. UBX domain

- Domain present in ubiquitin-regulatory proteins. Present in FAF1 and Shp1p. Number of
25 members: 19

[1] The UBA domain: a sequence motif present in multiple enzyme classes of the
ubiquitination pathway. Hofmann K, Bucher P; Trends Biochem Sci 1996;21:172-173.

- 30 681. (UCH) Ubiquitin carboxyl-terminal hydrolases family 1 cysteine active site
Ubiquitin carboxyl-terminal hydrolases (UCH) (deubiquitinating enzymes) [1,2] are thiol
proteases that recognize and hydrolyze the peptide bond at the C-terminal glycine of
ubiquitin. These enzymes are involved in the processing of poly-ubiquitin precursors as well
as that of ubiquitinated proteins. There are two distinct families of UCH. The first class consist

of enzymes of about 25 Kd and is currently represented by: - Mammalian isozymes L1 and L3. - Yeast YUH1. - Drosophila Uch. One of the active site residues of class-I UCH [3] is a cysteine. A signature pattern has been derived from the region around that residue.

Consensus pattern: Q-x(3)-N-[SA]-C-G-x(3)-[LIVM](2)-H-[SA]-[LIVM]-[SA] [C is the active site residue]

[1] Jentsch S., Seufert W., Hauser H.-P. *Biochim. Biophys. Acta* 1089:127-139(1991). [2] D'andrea A., Pellman D. *Crit. Rev. Biochem. Mol. Biol.* 33:337-352(1998). [3] Johnston S.C., Larsen C.N., Cook W.J., Wilkinson K.D., Hill C.P. *EMBO J.* 16:3787-3796(1997). [4] Rawlings N.D., Barrett A.J. *Meth. Enzymol.* 244:461-486(1994).

682. Ubiquitin carboxyl-terminal hydrolases family 2 signatures (UCH-1)

Ubiquitin carboxyl-terminal hydrolases (UCH) (deubiquitinating enzymes) [1,2] are thiol proteases that recognize and hydrolyze the peptide bond at the C-terminal glycine of ubiquitin. These enzymes are involved in the processing of poly-ubiquitin precursors as well as that of ubiquitinated proteins. There are two distinct families of UCH. The second class consist of large proteins (800 to 2000 residues) and is currently represented by: - Yeast UBP1, UBP2, UBP3, UBP4 (or DOA4/SSV7), UBP5, UBP7, UBP9, UBP10, UBP11, UBP12, UBP13, UBP14, UBP15 and UBP16. - Human tre-2. - Human isopeptidase T. - Human isopeptidase T-3. - Mammalian Ode-1. - Mammalian Unp. - Mouse Dub-1. - Drosophila fat facets protein (gene faf). - Mammalian faf homolog. - Drosophila D-Ubp-64E. - *Caenorhabditis elegans* hypothetical protein R10E11.3. - *Caenorhabditis elegans* hypothetical protein K02C4.3. These proteins only share two regions of similarity. The first region contains a conserved cysteine which is probably implicated in the catalytic mechanism. The second region contains two conserved histidines residues, one of which is also probably implicated in the catalytic mechanism. Signature patterns for both conserved regions have been developed.

Consensus pattern: G-[LIVMFY]-x(1,3)-[AGC]-[NASM]-x-C-[FYW]-[LIVMC]-[NST]-[SACV]-x-[LIVMS]-Q [C is the putative active site residue]

Consensus pattern: Y-x-L-x-[SAG]-[LIVMFT]-x(2)-H-x-G-x(4,5)-G-H-Y [The two H's are putative active site residues]

[1] Jentsch S., Seufert W., Hauser H.-P. *Biochim. Biophys. Acta* 1089:127-139(1991). [2] D'andrea A., Pellman D. *Crit. Rev. Biochem. Mol. Biol.* 33:337-352(1998). [3] Rawlings N.D., Barrett A.J. *Meth. Enzymol.* 244:461-486(1994).

683. Ubiquitin carboxyl-terminal hydrolases family 2 signatures (UCH-2)

Ubiquitin carboxyl-terminal hydrolases (UCH) (deubiquitinating enzymes) [1,2] are thiol

5 proteases that recognize and hydrolyze the peptide bond at the C-terminal glycine of ubiquitin. These enzymes are involved in the processing of poly-ubiquitin precursors as well as that of ubiquitinated proteins. There are two distinct families of UCH. The second class consist of largeproteins (800 to 2000 residues) and is currently represented by: - Yeast UBP1, UBP2, UBP3, UBP4 (or DOA4/SSV7), UBP5, UBP7, UBP9, UBP10, UBP11, UBP12, 10 UBP13, UBP14, UBP15 and UBP16. - Human tre-2. - Human isopeptidase T. - Human isopeptidase T-3. - Mammalian Ode-1. - Mammalian Unp. - Mouse Dub-1. - Drosophila fat facets protein (gene faf). - Mammalian faf homolog. - Drosophila D-Ubp-64E. - *Caenorhabditis elegans* hypothetical protein R10E11.3. - *Caenorhabditis elegans* hypothetical protein K02C4.3. These proteins only share two regions of similarity. The first region 15 contains a conserved cysteine which is probably implicated in the catalytic mechanism. The second region contains two conserved histidines residues, one of which is also probably implicated in the catalytic mechanism. Signature patterns for both conserved regions have been developed.

Consensus pattern: G-[LIVMFY]-x(1,3)-[AGC]-[NASM]-x-C-[FYW]-[LIVMC]-[NST]- 20 [SACV]-x-[LIVMS]-Q [C is the putative active site residue]

Consensus pattern: Y-x-L-x-[SAG]-[LIVMFT]-x(2)-H-x-G-x(4,5)-G-H-Y [The two H's are putative active site residues]

[1] Jentsch S., Seufert W., Hauser H.-P. *Biochim. Biophys. Acta* 1089:127-139(1991). [2] D'andrea A., Pellman D. *Crit. Rev. Biochem. Mol. Biol.* 33:337-352(1998). [3] Rawlings 25 N.D., Barrett A.J. *Meth. Enzymol.* 244:461-486(1994).

684. UDP-glycosyltransferases signature

UDP glycosyltransferases (UGT) are a superfamily of enzymes that catalyzes the addition of 30 the glycosyl group from a UTP-sugar to a small hydrophobic molecule. This family currently consist of: - Mammalian UDP-glucuronosyl transferases (UDPGT) [1,2]. A large family of membrane-bound microsomal enzymes which catalyze the transfer of glucuronic acid to a wide variety of exogenous and endogenous lipophilic substrates. These enzymes are of major importance in the detoxification and subsequent elimination of xenobiotics such as drugs and

carcinogens. - A large number of putative UDPGT from *Caenorhabditis elegans*. -

Mammalian 2-hydroxyacylsphingosine 1-beta-galactosyltransferase [3] (also known as UDP-galactose-ceramide galactosyltransferase). This enzyme catalyzes the transfer of galactose to ceramide, a key enzymatic step in the biosynthesis of galactocerebrosides, which are

5 abundant sphingolipids of the myelin membrane of the central nervous system and peripheral nervous system. - Plants flavonol O(3)-glucosyltransferase. An enzyme [4] that catalyzes the transfer of glucose from UDP-glucose to a flavanol. This reaction is essential and one of the last steps in anthocyanin pigment biosynthesis. - Baculoviruses ecdysteroid UDP-glucosyltransferase (EC 2.4.1.-) [5] (egt). This enzyme catalyzes the transfer of glucose from

10 UDP-glucose to ecdysteroids which are insect molting hormones. The expression of egt in the insect host interferes with the normal insect development by blocking the molting process. -

Prokaryotic zeaxanthin glucosyl transferase (gene crtX), an enzyme involved in carotenoid biosynthesis and that catalyses the glycosylation reaction which converts zeaxanthin to zeaxanthin-beta- diglucoside. - Streptomyces macrolide glycosyltransferases [6]. These

15 enzymes specifically inactivates macrolide antibiotics via 2'-O-glycosylation using UDP-glucose. These enzymes share a conserved domain of about 50 amino acid residues located in their C-terminal section and from which a pattern has been extracted to detect them.

Consensus pattern: [FW]-x(2)-Q-x(2)-[LIVMYA]-[LIMV]-x(4,6)-[LVGAC]-[LVFYA]-[LIVMF]-[STAGCM]-[HNQ]-[STAGC]-G-x(2)-[STAG]-x(3)-[STAGL]-[LIVMFA]-x(4)-

20 [PQR]-[LIVMT]-x(3)-[PA]-x(3)-[DES]-[QEHN]

[1] Dutton G.J. (In) Glucuronidation of drugs and other compounds, Dutton G.J., Ed., pp 1-78, CRC Press, Boca Raton, (1980). [2] Burchell B., Nebert D.W., Nelson D.R., Bock K.W., Iyanagi T., Jansen P.L., Lancet D., Mulder G.J., Chowdhury J.R., Siest G., Tephly T.R., Mackenzie P.I. DNA Cell Biol. 10:487-494(1991). [3] Schulte S., Stoffel W. Proc. Natl. Acad. Sci. U.S.A. 90:10265-10269(1993). [4] Furtek D., Schiefelbein J.W., Johnston F., Nelson O.E. Jr. Plant Mol. Biol. 11:473-481(1988). [5] O'Reilly D.R., Miller L.K. Science 245:1110-1112(1989). [6] Hernandez C., Olano C., Mendez C., Salas J.A. Gene 134:139-140(1993).

685. UDP-glucose/GDP-mannose dehydrogenase family

The UDP-glucose/GDP-mannose dehydrogenases are a small group of enzymes which possesses the ability to catalyze the NAD-dependent 2-fold oxidation of an alcohol to an acid without the release of an aldehyde intermediate [2]. Number of members: 55

[1] Purification and characterization of guanosine diphospho-D-mannose dehydrogenase. A key enzyme in the biosynthesis of alginate by *Pseudomonas aeruginosa*. Roychoudhury S, May TB, Gill JF, Singh SK, Feingold DS, Chakrabarty AM; J Biol Chem 1989;264:9380-9385. [2] Properties and kinetic analysis of UDP-glucose dehydrogenase from group A streptococci. Irreversible inhibition by UDP-chloroacetol. Campbell RE, Sala RF, van de Rijn I, Tanner ME; J Biol Chem 1997;272:3416-3422.

686. Uracil-DNA glycosylase signature

Uracil-DNA glycosylase (EC 3.2.2.-) (UNG) [1] is a DNA repair enzyme that excises uracil residues from DNA by cleaving the N-glycosylic bond. Uracil in DNA can arise as a result of misincorporation of dUMP residues by DNA polymerase or deamination of cytosine. The sequence of uracil-DNA glycosylase is extremely well conserved [2] in bacteria and eukaryotes as well as in herpes viruses. More distantly related uracil-DNA glycosylases are also found in poxviruses [3]. In eukaryotic cells, UNG activity is found in both the nucleus and the mitochondria. Human UNG1 protein is transported to both the mitochondria and the nucleus [4]. The N-terminal 77 amino acids of UNG1 seem to be required for mitochondrial localization [4], but the presence of a mitochondrial transitpeptide has not been directly demonstrated. As a signature for this type of enzyme, the most N-terminal conserved region has been selected. This region contains an aspartic acid residue which has been proposed, based on X-ray structures [5,6] to act as a general base in the catalytic mechanism. Consensus pattern: [KR]-[LIV]-[LIVC]-[LIVM]-x-G-[QT]-D-P-Y [D is the active site residue]-

[1] Sancar A., Sancar G.B. Annu. Rev. Biochem. 57:29-67(1988). [2] Olsen L.C., Aasland R., Wittwer C.U., Krokan H.E., Helland D.E. EMBO J. 8:3121-3125 (1989). [3] Upton C., Stuart D.T., McFadden G. Proc. Natl. Acad. Sci. U.S.A. 90:4518-4522(1993). [4] Slupphaug G., Markussen F.-H., Olsen L.C., Aasland R., Aarsaether N., Bakke O., Krokan H.E., Helland D.E. Nucleic Acids Res. 21:2579-2584(1993). [5] Savva R., McAuley-Hecht K., Brown T., Pearl L. Nature 373:487-493(1995). [6] Mol C.D., Arvai A.S., Slupphaug G., Kavli B., Alseth I., Krokan H.E., Tainer J.A. Cell 80:869-878(1995). [7] Muller S.J., Caradonna S. Biochim. Biophys. Acta 1088:197-207(1991). [8] Meyer-Siegler K., Mauro D.J., Seal G., Wurzer J., Deriel J.K., Sirover M.A. Proc. Natl. Acad. Sci. U.S.A. 88:8460-8464(1991). [9] Muller S.J., Caradonna S. J. Biol. Chem. 268:1310-1319(1993). [10] Barnes D.E., Lindahl T., Sedgwick B. Curr. Opin. Cell Biol. 5:424-433(1993).

687. Uncharacterized protein family UPF0001 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: -

- 5 Yeast chromosome II hypothetical protein YBL036c. - *Caenorhabditis elegans* hypothetical protein F09E5.8. - *Bacillus subtilis* hypothetical protein ylmE. - *Escherichia coli* hypothetical protein yggS and HI0090, the corresponding *Haemophilus influenzae* protein. - *Helicobacter pylori* hypothetical protein HP0395. - *Mycobacterium tuberculosis* hypothetical protein MtCY270.20. - *Synechocystis* strain PCC 6803 hypothetical protein slr0556. - A
- 10 *Pseudomonas aeruginosa* hypothetical protein in pilT 5' region. - A *Vibrio alginolyticus* hypothetical protein in pilT 5' region. These are proteins of from 25 to 30 Kd which contain a number of conserved regions. The best conserved region which is located in the first third of these proteins has been selected as a signature pattern.

Consensus pattern: [FW]-H-[FM]-[IV]-G-x-[LIV]-Q-x-[NKR]-K-x(3)-[LIV]

- 15 [1] Bairoch A., Rudd K.E. Unpublished observations (1996).

688. Uncharacterized protein family UPF0003 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: -

- 20 *Escherichia coli* protein aefA. - *Escherichia coli* hypothetical protein yggB. - *Escherichia coli* hypothetical protein yjeP and HI0195.1, the corresponding *Haemophilus influenzae* protein. - *Escherichia coli* hypothetical protein ynaI. - *Bacillus subtilis* hypothetical protein yhdY. - *Helicobacter pylori* hypothetical protein HP0415. - *Synechocystis* strain PCC 6803 hypothetical protein slr0639. - *Archaeoglobus fulgidus* hypothetical protein AF1546. -
- 25 *Methanococcus jannaschii* hypothetical protein MJ0170. - *Methanococcus jannaschii* hypothetical protein MJ1143. The size of these proteins range from 30 to 120 Kd. They all contain a number of transmembrane regions. The best conserved region which is located in and just after the last potential transmembrane region has been selected as a signature pattern.,

- 30 Consensus pattern: G-[STIF]-V-x(2)-[LIVM]-x(6)-[LIVMF]-x(3)-[DQ]-x(3)-[LIV]-x-[LIV]-P-N-x(2)-[LIVMF]-[LIVFSTA]-x(5)-N

[1] Bairoch A. Unpublished observations (1997).

689. Uncharacterized protein family UPF0004 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - *Escherichia coli* hypothetical protein yliG. - *Escherichia coli* hypothetical protein yleA and HI0019, the corresponding *Haemophilus influenzae* protein. - *Bacillus subtilis* hypothetical protein yqeV. - *Helicobacter pylori* hypothetical protein HP0269. - *Helicobacter pylori* hypothetical protein HP0285. - *Mycoplasma iowae* hypothetical protein in 16S RNA 5'region. - *Mycobacterium leprae* hypothetical protein B2235_C2_195. - *Pseudomonas aeruginosa* hypothetical protein in hemL 3'region. - *Synechocystis* strain PCC 6803 hypothetical protein slr0082. - *Synechocystis* strain PCC 6803 hypothetical protein slI0996. - *Methanococcus jannaschii* hypothetical protein MJ0865. - *Methanococcus jannaschii* hypothetical protein MJ0867. - *Caenorhabditis elegans* hypothetical protein F25B5.5. The size of these proteins range from 47 to 61 Kd. They contain six conserved cysteines, three of which are clustered in a region that can be used as signature pattern.

Consensus pattern: [LIVM]-x-[LIVMT]-x(2)-G-C-x(3)-C-[STAN]-[FY]-C-x-[LIVM]- x(4)-G

[1] Bairoch A. Unpublished observations (1997).

690. Uncharacterized protein family UPF0005 signature

The following proteins seems to be evolutionary related [1]: - Mammalian protein TEGT (Testis Enhanced Gene Transcript). - *Escherichia coli* hypothetical protein yccA and HI0044, the corresponding *Haemophilus influenzae* protein. - A probable *Pseudomonas aeruginosa* ortholog of yccA. These are proteins of about 25 Kd which seem to contain seven transmembranedomains. A signature pattern that corresponds to a region that starts with the beginning of the third transmembrane domain and ends in the middle of the fourth one has been developed.

Consensus pattern: G-[LIVM](2)-[SA]-x(5,8)-G-x(2)-[LIVM]-G-P-x-L-x(4)-[SAG]- x(4,6)-[LIVM](2)-x(2)-A-x(3)-T-A-[LIVM](2)-F

[1] Walter L., Marynen P., Szpirer J., Levan G., Guenther E. Genomics 28:301-304(1995).

691. Uncharacterized protein family UPF0006 signatures

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast chromosome II hypothetical protein YBL055c. - *Escherichia coli* hypothetical protein

ycfH and HI0454, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein yigW. - Escherichia coli hypothetical protein yijV and HI0081, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein yabD. - Haemophilus influenzae hypothetical protein HI1664. - Mycoplasma genitalium

- 5 hypothetical protein MG009. These are proteins of from 24 to 47 Kd which contain a number of conserved regions. They can be picked up in the database by the following patterns.

Consensus pattern: [LIVMFY](2)-D-[STA]-H-x-H-[LIVMF]-[DN

Consensus pattern: P-[LIVM]-x-[LIVM]-H-x-R-x-[TA]-x-[DE

Consensus pattern: [LVSA]-[LIVA]-x(2)-[LIVM]-[PS]-x(3)-L-[LIVM]-[LIVMS]-E-T- D-x-

10 P

[1] Bairoch A., Rudd K.E. Unpublished observations (1995).

692. Uncharacterized protein family UPF0007 signature

- 15 The following proteins seems to be evolutionary related [1]: - Escherichia coli hypothetical protein ygbP and HI0672, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein yacM. - Mycobacterium tuberculosis hypothetical protein MitCY06G11.29c. - Synechocystis strain PCC 6803 hypothetical protein slr0951. - A Rhodobacter capsulatus hypothetical protein in nifR3 5'region. Except for the Rhodobacter
- 20 protein which contains a C-terminal extension, all these proteins have from 225 to 236 amino acids. They are hydrophilic proteins that can be picked up in the database by the following pattern.

Consensus pattern: V-L-[IV]-H-D-[GA]-A-R

[1] Bairoch A. Unpublished observations (1997).

25

693. Uncharacterized protein family UPF0015 signature

- The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast chromosome II hypothetical protein YBR002c. - Yeast chromosome XIII hypothetical
- 30 protein YMR101c. - Escherichia coli hypothetical protein yaeU and HI0920, the corresponding Haemophilus influenzae protein. - Helicobacter pylori hypothetical protein HP1221. - Mycobacterium leprae hypothetical protein B1937_F2_65. - A Corynebacterium glutamicum hypothetical protein in aroF 3'region. - A Streptomyces fradiae hypothetical protein in transposon Tn4556. - Synechocystis strain PCC 6803 hypothetical protein slI0505.

- *Methanococcus jannaschii* hypothetical protein MJ1372. These are proteins of about 26 to 40 Kd whose central region is well conserved. They can be picked up in the database by the following pattern.

Consensus pattern: [DE]-[LIVMF](3)-R-T-[SG]-G-x(2)-R-x-S-x-[FY]-[LIVM](2)-W-Q-

- 5 [1] Wolfe K.H., Lohan A.J.E. Yeast 10:S41-S46(1994).

694. Uncharacterized protein family UPF0016 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: -

- 10 Yeast hypothetical protein YBR187w. - Fission yeast hypothetical protein SpAC17G8.08c. -
Mouse protein pFT27. - *Synechocystis* strain PCC 6803 hypothetical protein sl10615. These
are hydrophobic proteins of 200 to 320 amino acids that seem to contain six or seven
transmembrane domains. A conserved region which seems, in the eukaryotic proteins of this
family, to directly follow the second transmembrane domain has been selected as a signature
15 pattern.

Consensus pattern: E-[LIVM]-G-D-K-T-F-[LIVMF](2)-A-

- [1] Bairoch A. Unpublished observations (1996).

20 695. Uncharacterized protein family UPF0021 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: -

- Yeast chromosome VII hypothetical protein YGL211w. - *Dictyostelium discoideum* protein
veg136. - *Methanococcus jannaschii* hypothetical proteins MJ1157 and MJ1478. These are
proteins of from 300 to 360 residues. They can be picked up in the database by the following
25 pattern which is located in their N-terminal section.

Consensus pattern: C-K-x(2)-F-x(4)-E-x(22,23)-S-G-G-K-D

- [1] Bairoch A. Unpublished observations (1997).

30 696. Uncharacterized protein family UPF0023 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: -

- Mouse protein 22A3. - Yeast chromosome XII hypothetical protein YLR022c. -
Caenorhabditis elegans hypothetical protein W06E11.4. - *Methanococcus jannaschii*

hypothetical protein MJ0592. These are hydrophilic proteins of about 30 Kd. They can be picked up in the database by the following pattern.

Consensus pattern: D-x-D-E-[LIV]-L-x(4)-V-F-x(3)-S-K-G-

[1] Bairoch A. Unpublished observations (1997).

5

697. Uncharacterized protein family UPF0024 signature. The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein ygbO and HI0701, the corresponding Haemophilus influenzae protein. - Helicobacter pylori hypothetical protein HP0926. - Yeast chromosome XV hypothetical protein YOR243c. - Caenorhabditis elegans hypothetical protein B0024.11. - Methanococcus jannaschii hypothetical proteins MJ0588 and MJ1364. These are hydrophilic proteins of from 39 to 77 Kd. They can be picked up in the database by the following pattern.

10

15

Consensus pattern: G-x-K-D-[KR]-x-A-[LV]-T-x-Q-x-[LIVF]-[SGC]-

[1] Bairoch A. Unpublished observations (1997).

20

698. Uncharacterized protein family UPF0025 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein yfcE. - Bacillus subtilis hypothetical protein ysnB. - Mycoplasma genitalium and pneumoniae hypothetical protein MG207. - Methanococcus jannaschii hypothetical proteins MJ0623 and MJ0936. These are hydrophilic proteins of about 20 Kd. They can be picked up in the database by the following pattern.

25

Consensus pattern: D-V-[LIV]-x(2)-G-H-[ST]-H-x(12)-[LIVMF]-N-P-G

[1] Bairoch A. Unpublished observations (1997).

30

699. Uncharacterized protein family UPF0029 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast chromosome III hypothetical protein YCR59c. - Yeast chromosome IV hypothetical protein YDL177C. - Escherichia coli hypothetical protein yigZ and HI0722, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein yvyE.

- A *Thermus aquaticus* hypothetical protein in pol 5' region. These proteins can be picked up in the database by the following pattern.

Consensus pattern: G-x(2)-[LIVM](2)-x(2)-[LIVM]-x(4)-[LIVM]-x(5)-[LIVM](2)-x- R-[FYW](2)-G-G-x(2)-[LIVM]-G

- 5 [1] Koonin E.V., Bork P., Sander C. EMBO J. 13:493-503(1994).

700. Uncharacterized protein family UPF0030 signature

The following uncharacterized proteins have been shown [1] to be highly similar: - Yeast

- 10 chromosome VI hypothetical protein YFL060c. - Yeast chromosome XIII hypothetical protein YMR095c. - Yeast chromosome XIV hypothetical protein YNL334c. - *Bacillus subtilis* hypothetical protein yaaE. - *Haemophilus influenzae* hypothetical protein HI1648. - *Methanococcus jannaschii* hypothetical protein MJ1661. These are hydrophilic proteins of about 19 to 25 Kd. They can be picked up in the database by the following pattern.

- 15 Consensus pattern: [GA]-L-I-[LIV]-P-G-G-E-S-T-[STA]

[1] Bairoch A. Unpublished observations (1997).

701. Uncharacterized protein family UPF0032 signature

- 20 The following uncharacterized proteins have been shown [1] to share regions of similarities: - *Escherichia coli* hypothetical protein yigU and HI0188, the corresponding *Haemophilus influenzae* protein. - *Bacillus subtilis* hypothetical protein ycbT. - *Mycobacterium tuberculosis* hypothetical protein MtCY49.33c and U2126A, the corresponding *Mycobacterium leprae* protein. - *Synechocystis* strain PCC 6803 hypothetical protein sl10194.
- 25 - *Odontella sinensis* and *Porphyra purpurea* chloroplast hypothetical protein ycf43. These proteins have from 245 to 317 amino acids and seem to contain at least six or seven transmembrane regions. A conserved region located in the central section of these proteins has been developed as a signature pattern.,

Consensus pattern: Y-x(2)-F-[LIVMA](2)-x-L-x(4)-G-x(2)-F-[EQ]-[LIVMF]-P- [LIVM] -

- 30 [1] Bairoch A., Rudd K.E. Unpublished observations (1996).

702. Uncharacterized protein family UPF0034 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein yhdG and HI0979, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein yjbN and HI0634, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein yohI and HI0270, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein yacF. - Rhodobacter capsulatus protein nifR3 and related proteins in Azospirillum brasilense and Rhizobium leguminosarum. - Synechocystis strain PCC 6803 hypothetical protein slr0644. - Synechocystis strain PCC 6803 hypothetical protein slI0926. - Caenorhabditis elegans hypothetical protein C45G9.2. - Yeast protein SMM1. - Yeast hypothetical protein YLR401c. - Yeast hypothetical protein YLR405w. - Yeast hypothetical protein YML080w. Although it has been proposed [2] that Rhodobacter capsulatus nifR3 is a transcriptional regulatory protein, it is believed that these proteins constitute a family of enzymes whose active site could include a conserved cysteine which has been used as the central part of a signature pattern.

Consensus pattern: [LIVM]-[DNG]-[LIVM]-N-x-G-C-P-x(3)-[LIVMASO]-x(5)-G-[SAC] [1] Bairoch A., Rudd K.E. Unpublished observations (1995).[2] Foster-Hartnett D., Cullen P.J., Gabbert K.K., Kranz R.G. Mol. Microbiol. 8:903-914(1993).

703. Uncharacterized protein family UPF0038 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein yacE and HI0890, the corresponding Haemophilus influenzae protein. - Mycobacterium tuberculosis hypothetical protein MtCY01B2.23 and O410, the corresponding Mycobacterium leprae protein. - Synechocystis strain PCC 6803 hypothetical protein slr0553. - Other hypothetical proteins from Aeromonas hydrophila, Bacteroides nodosus, Neisseria gonorrhoeae, Pseudomonas putida, Thermus thermophilus and Xanthomonas campestris. - Human hypothetical protein pOV-2. - Yeast hypothetical protein YDR196C. - Caenorhabditis elegans hypothetical protein T05G5.5. These proteins all contain, in their N-terminal extremity, an ATP/GTP-binding motif 'A' (P-loop) (see <PDOC00017>). The size of these proteins range from 200 to 290 residues (with the exception of the Mycobacterial sequences which are 410 residues long). A conserved region some 50 residues away from the ATP-binding P-loop has been developed as a signature pattern.

Consensus pattern: G-x-[LI]-x-R-x(2)-L-x(4)-F-x(8)-[LIV]-x(5)-P-x-[LIV]-

[1] Rudd K.E., Bairoch A. Unpublished observations (1997).

704. Ubiquitin-conjugating enzymes active site

- 5 Ubiquitin-conjugating enzymes (UBC or E2 enzymes) [1,2,3] catalyze the covalent attachment of ubiquitin to target proteins. An activatedubiquitin moiety is transferred from an ubiquitin-activating enzyme (E1) to E2which later ligates ubiquitin directly to substrate proteins with or without the assistance of 'N-end' recognizing proteins (E3). In most species there are many forms of UBC (at least 9 in yeast) which are implicated in diverse cellular
- 10 functions. A cysteine residue is required for ubiquitin-thiolester formation. There is a single conserved cysteine in UBC's and the region around that residue is conserved in the sequence of known UBC isozymes. That region has been used as a signature pattern.
- Consensus pattern: [FYWLSP]-H-[PC]-[NH]-[LIV]-x(3,4)-G-x-[LIV]-C-[LIV]-x- [LIV] [C is the active site residue]

- 15 [1] Jentsch S., Seufert W., Sommer T., Reins H.-A. Trends Biochem. Sci. 15:195-198(1990).[2] Jentsch S., Seufert W., Hauser H.-P. Biochim. Biophys. Acta 1089:127-139(1991).[3] Hershko A. Trends Biochem. Sci. 16:265-268(1991).

20 705. Uroporphyrinogen decarboxylase signatures

- Uroporphyrinogen decarboxylase (URO-D), the fifth enzyme of the heme biosynthetic pathway, catalyzes the sequential decarboxylation of the four acetyl side chains of uroporphyrinogen to yield coproporphyrinogen [1].URO-D deficiency is responsible for the Human genetic diseases familialporphyria cutanea tarda (fPCT) and hepatoerythropoietic
- 25 porphyria (HEP).The sequence of URO-D has been well conserved throughout evolution. The best conserved region is located in the N-terminal section; it contains a perfectlyconserved hexapeptide. There are two arginine residues in this hexapeptide which could be involved in the binding, via salt bridges, to the carboxylgroups of the propionate side chains of the substrate. This region has been used as a signature pattern. A second
- 30 signature pattern is based on a another well conserved region which is located in the central section of the protein.

Consensus pattern: P-x-W-x-M-R-Q-A-G-R

Consensus pattern: G-F-[STAGCV]-[STAGC]-x-P-[FYW]-T-[LV]-x(2)-Y-x(2)-[AE]- [GK]

[1] Garey J.R., Labbe-Bois R., Chelstowska A., Rytka J., Harrison L., Kushner J., Labbe P.
Eur. J. Biochem. 205:1011-1016(1992).

5 706. ubiE/COQ5 methyltransferase family signatures

The following methyltransferases have been shown [1] to share regions of similarities: -
Escherichia coli ubiE, which is involved in both ubiquinone and menaquinone biosynthesis
and which catalyzes the S-adenosylmethionine dependent methylation of 2-polyprenyl-6-
methoxy-1,4-benzoquinol into 2-polyprenyl-3-methyl-6-methoxy-1,4-benzoquinol and of
10 demethylmenaquinol into menaquinol. - Yeast COQ5, a ubiquinone biosynthesis
methyltransferase. - Bacillus subtilis spore germination protein C2 (gene: gercB or gerC2), a
probable menaquinone biosynthesis methyltransferase. - Lactococcus lactis gerC2 homolog. -
Caenorhabditis elegans hypothetical protein ZK652.9. - Leishmania donovani amastigote-
specific protein A41. These are hydrophilic proteins of about 30 Kd (except for ZK652.9
15 which is 65Kd). They can be picked up in the database by the following patterns.
Consensus pattern: Y-D-x-M-N-x(2)-[LIVM]-S-x(3)-H-x(2)-W
Consensus pattern: R-V-[LIVM]-K-[PV]-G-G-x-[LIVMF]-x(2)-[LIVM]-E-x-S
[1] Lee P.T., Hsu A.Y., Ha H.T., Clarke C.F. J. Bacteriol. 179:1748-1754(1997).

20

707. Uricase signature

Uricase (urate oxidase) [1] is the peroxisomal enzyme responsible for the degradation of
urate into allantoin. Some species, like primates and birds, have lost the gene for uricase and
are therefore unable to degrade urate. Uricase is a protein of 300 to 400 amino acids. A highly
25 conserved region located in the central part of the sequence has been used as a signature
pattern.

Consensus pattern: [LV]-x-[LV]-[LIV]-K-[STV]-[ST]-x-[SN]-x-F-x(2)-[FY]-x(4)-[FY]-
x(2)-L-x(5)-R

[1] Motojima K., Kanaya S., Goto S. J. Biol. Chem. 263:16677-16681(1988).

30

708. Universal stress protein family (Usp)

By a wide range of stress conditions members of the Usp family are predicted to be related to the MADS-box proteins transcript_fact and bind to DNA [2]. Number of members:

39

- 5 [1] Expression and role of the universal stress protein, UspA, of Escherichia coli during growth arrest. Nystrom T, Neidhardt FC; Mol Microbiol 1994; 11:537-544.
[2] Sequence analysis of eukaryotic developmental proteins: ancient and novel domains. Mushegian AR, Koonin EV; Genetics 1996; 144:817-828.

10

709. Ubiquitin domain signature and profile

- Ubiquitin [1,2,3] is a protein of seventy six amino acid residues, found in all eukaryotic cells and whose sequence is extremely well conserved from protozoan to vertebrates. It plays a key role in a variety of cellular processes, such as ATP-dependent selective degradation of cellular proteins, maintenance of chromatin structure, regulation of gene expression, stress response and ribosome biogenesis. In most species, there are many genes coding for ubiquitin. However they can be classified into two classes. The first class produces polyubiquitin molecules consisting of exact head to tail repeats of ubiquitin. The number of repeats is variable (up to twelve in a Xenopus gene). In the majority of polyubiquitin precursors, there is a final amino-acid after the last repeat. The second class of genes produces precursor proteins consisting of a single copy of ubiquitin fused to a C-terminal extension protein (CEP). There are two types of CEP proteins and both seem to be ribosomal proteins. Ubiquitin is a globular protein, the last four C-terminal residues (Leu-Arg- Gly-Gly) extending from the compact structure to form a 'tail', important for its function. The latter is mediated by the covalent conjugation of ubiquitin to target proteins, by an isopeptide linkage between the C-terminal glycine and the epsilon amino group of lysine residues in the target proteins. There are a number of proteins which are evolutionary related to ubiquitin: - Ubiquitin-like proteins from baculoviruses as well as in some strains of bovine viral diarrhea viruses (BVDV). These proteins are highly similar to their eukaryotic counterparts. - Mammalian protein GDX [4]. GDX is composed of two domains, a N-terminal ubiquitin-like domain of 74 residues and a C-terminal domain of 83 residues with some similarity with the thyroglobulin hormonogenic site. - Mammalian protein FAU [5]. FAU is a fusion protein which consist of a N-terminal ubiquitin-like protein of 74 residues fused to ribosomal protein S30. - Mouse protein NEDD-8 [6], a ubiquitin-like protein of 81 residues. - Human protein

BAT3, a large fusion protein of 1132 residues that contains a N-terminal ubiquitin-like domain. - *Caenorhabditis elegans* protein ubl-1 [7]. Ubl-1 is a fusion protein which consist of a N-terminal ubiquitin-like protein of 70 residues fused to ribosomal protein S27A. - Yeast DNA repair protein RAD23 [8]. RAD23 contains a N-terminal domain that seems to be

5 distantly, yet significantly, related to ubiquitin. - Mammalian RAD23-related proteins RAD23A and RAD23B. - Mammalian BCL-2 binding athanogene-1 (BAG-1). BAG-1 is a protein of 274 residues that contains a central ubiquitin-like domain. - Human spliceosome associated protein 114 (SAP 114 or SF3A120). - Yeast protein DSK2, a protein involved in spindle pole body duplication and which contains a N-terminal ubiquitin-like domain. -

10 Human protein CKAP1/TFCB, *Schizosaccharomyces pombe* protein alp11 and *Caenorhabditis elegans* hypothetical protein F53F4.3. These proteins contain a N-terminal ubiquitin domain and a C-terminal CAP-Gly domain. - *Schizosaccharomyces pombe* hypothetical protein SpAC26A3.16. This protein contains a N-terminal ubiquitin domain. - Yeast protein SMT3. - Human ubiquitin-like proteins SMT3A and SMT3B. - Human

15 ubiquitin-like protein SMT3C (also known as PIC1; Ubl1, Sumo-1; Gmp-1 or Sentrin). This protein is involved in targeting ranGAP1 to the nuclear pore complex protein ranBP2. - SMT3-like proteins in plants and *Caenorhabditis elegans*. To identify ubiquitin and related proteins, a pattern has been developed based on conserved positions in the central section of the sequence. A profile was also developed that spans the complete length of the ubiquitin

20 domain.
Consensus pattern: K-x(2)-[LIVM]-x-[DESAK]-x(3)-[LIVM]-[PA]-x(3)-Q-x-[LIVM]-[LIVMC]-[LIVMFY]-x-G-x(4)-[DE]

[1] Jentsch S., Seufert W., Hauser H.-P. *Biochim. Biophys. Acta* 1089:127-139(1991).[2] Monia B.P., Ecker D.J., Croke S.T. *Bio/Technology* 8:209-215(1990).[3] Finley D.,

25 Varshavsky A. *Trends Biochem. Sci.* 10:343-347(1985).[4] Filippi M., Tribioli C., Toniolo D. *Genomics* 7:453-457(1990).[5] Olvera J., Wool I.G. *J. Biol. Chem.* 268:17967-17974(1993).[6] Kumar S., Yoshida Y., Noda M. *Biochem. Biophys. Res. Commun.* 195:393-399(1993).[7] Jones D., Candido E.P. *J. Biol. Chem.* 268:19545-19551(1993).[8] Melnick L., Sherman F. *J. Mol. Biol.* 233:372-388(1993).

30
710. VHS domain

Domain present in VPS-27, Hrs and STAM. Number of members: 27

711. Vinculin family signatures

Vinculin [1] is a eukaryotic protein that seems to be involved in the attachment of the actin-based microfilaments to the plasma membrane. Vinculin is located at the cytoplasmic side of focal contacts or adhesion plaques. In addition to actin, vinculin interacts with other structural proteins such as talin and alpha-actinins. Vinculin is a large protein of 116 Kd (about a 1000 residues). Structurally the protein consists of an acidic N-terminal domain of about 90 Kd separated from a basic C-terminal domain of about 25 Kd by a proline-rich region of about 50 residues. The central part of the N-terminal domain consists of a variable number (3 in vertebrates, 2 in *Caenorhabditis elegans*) of repeats of a 110 amino acids domain. Catenins [2] are proteins that associate with the cytoplasmic domain of a variety of cadherins. The association of catenins to cadherins produces a complex which is linked to the actin filament network, and which seems to be of primary importance for cadherins cell-adhesion properties. Three different types of catenins seem to exist: alpha, beta, and gamma. Alpha-catenins are proteins of about 100 Kd which are evolutionarily related to vinculin. In terms of their structure the most significant differences are the absence, in alpha-catenin, of the repeated domain and of the proline-rich segment. Two signature patterns for this family of proteins have been developed. The first pattern is located in the N-terminal section of both vinculin and alpha-catenins and is part, in vinculin, of a domain that seems to be involved with the interaction with talin. The second pattern is based on a conserved region in the N-terminal part of the repeated domain of vinculin.

Consensus pattern: [KR]-x-[LIVMF]-x(3)-[LIVMA]-x(2)-[LIVM]-x(6)-R-Q-Q-E-L

Consensus pattern: [LIVM]-x-[QA]-A-x(2)-W-[IL]-x-[DN]-P

[1] Otto J.J. Cell Motil. Cytoskeleton 16:1-6(1990). [2] Herrenknecht K., Ozawa M.,

Eckerskorn C., Lottspeich F., Lenter M., Kemler R. Proc. Natl. Acad. Sci. U.S.A. 88:9156-9160(1991).

712. (Vitellogenin N) Lipoprotein amino terminal region

This family contains regions from: Vitellogenin, Microsomal triglyceride transfer protein and apolipoprotein B-100. These proteins are all involved in lipid transport [1]. This family contains the LV1n chain from lipovitellin, that contains two structural domains.

Number of members: 33

[1] The structural basis of lipid interactions in lipovitellin, a soluble lipoprotein.
Anderson TA, Levitt DG, Banaszak LJ Structure 1998;6:895-909.

5 713. (VMSA) Major surface antigen from hepadnavirus

714. ssDNA binding protein (Viral DNA bp)

This protein is found in herpesviruses and is needed for
10 replication.

715. (Votage CLC) Voltage gated chloride channels

15 This family of ion channels contains 10 or 12 transmembrane helices. Each protein forms a single pore. It has been shown that some members of this family form homodimers. These proteins contain two CBS domains.

[1] Schmidt-Rose T, Jentsch TJ; J Biol Chem 1997;272:20515-20521.

20 [2] Zhang J, George AL Jr, Griggs RC, Fouad GT, Roberts J, Kwiecinski H, Connolly AM, Ptacek LJ; Neurology 1996;47:993-998.

716. von Willebrand factor type A domain (vwa)

25 More von Willebrand factor type A domains? Sequence similarities with malaria thrombospondin-related anonymous protein, dihydropyridine-sensitive calcium channel and inter-alpha-trypsin inhibitor.

Bork P, Rohde K;

30 Biochem J 1991;279:908-911.

1. RUGGERI, Z.M. and WARE, J.

von Willebrand factor.

FASEB J. 7 308-316 (1993).

2. COLOMBATTI, A., BONALDO, P. and DOLIANA, R.

Type A modules: interacting domains found in several non-fibrillar collagens and in other extracellular matrix proteins.

5 MATRIX 13 297-306 (1993).

3. PERKINS, S.J., SMITH, K.F., WILLIAMS, S.C., HARIS, P.I., CHAPMAN, D. and SIM, R.B.

10 The secondary structure of the von Willebrand factor type A domain in factor B of human complement by Fourier transform infrared spectroscopy. Its occurrence in collagen types VI, VII, XII and XIV, the integrins and other proteins by averaged structure predictions.

J.MOL.BIOL. 238 104-119 (1994).

15 4. BORK, P. and ROHDE, K.

More von Willebrand factor type A domains? Sequence similarities with malaria thrombospondin-related anonymous protein, dihydropyridine-sensitive calcium channel and inter-alpha-trypsin inhibitor.

BIOCHEM.J. 279 908-910 (1991).

20 5. EDWARDS, Y.J.K. and PERKINS, S.J.

The protein fold of the von Willebrand factor type A domain is predicted to be similar to the open twisted beta-sheet flanked by alpha-helices found in human ras-p21.

25 FEBS LETT. 358 283-286 (1995).

6. LEE, J.O., RIEU, P., ARNAOUT, M.A. and LIDDINGTON, R.

Crystal structure of the A domain from the alpha subunit of integrin CR3 (CD11b/CD18).

30 CELL 80 631-638 (1995).

7. QU, A. and LEAHY, D.J.

Crystal structure of the I-domain from the CD11a/CD18 (LFA-1, alpha L beta 2) integrin.

The von Willebrand factor is a large multimeric glycoprotein found in blood plasma. Mutant forms are involved in the aetiology of bleeding disorders [1]. In von Willebrand factor, the type A domain (vWF) is the prototype for a protein superfamily. The vWF domain is found in various plasma proteins: complement factors B, C2, CR3 and CR4; the integrins (I-domains); collagen types VI, VII, XII and XIV; and other extracellular proteins [2-4]. Proteins that incorporate vWF domains participate in numerous biological events (e.g., cell adhesion, migration, homing, pattern formation, and signal transduction), involving interaction with a large array of ligands [2]. Secondary structure prediction from 75 aligned vWF sequences has revealed a largely alternating sequence of alpha-helices and beta-strands [3]. Fold recognition algorithms were used to score sequence compatibility with a library of known structures: the vWF domain fold was predicted to be a doubly-wound, open, twisted beta-sheet flanked by alpha-helices [5]. 3D structures have been determined for the I-domains of integrins CD11b (with bound magnesium) [6] and CD11a (with bound manganese) [7]. The domain adopts a classic alpha/beta Rossmann fold and contains an unusual metal ion coordination site at its surface. It has been suggested that this site represents a general metal ion-dependent adhesion site (MIDAS) for binding protein ligands [6]. The residues constituting the MIDAS motif in the CD11b and CD11a I-domains are completely conserved, but the manner in which the metal ion is coordinated differs slightly [7].

VWFADOMAIN is a 3-element fingerprint that provides a signature for the vWF domain superfamily. The fingerprint was derived from an initial alignment of 14 sequences. Motif 1 includes the first beta-strand and 3 conserved residues involved in metal ion coordination in I-domains (Asp and 2 serines in positions 8, 10 and 12, respectively); motif 2 spans strands beta-2 and beta-2'; and motif 3 encodes beta-strand 3 and a conserved Asp (in position 7), which coordinates the metal ion [6,7]. Three iterations on OWL27.0 were required to reach convergence, at which point a true set comprising 56 sequences was identified. Numerous partial matches were also found.

717. (WD40) WD domain, G-beta repeat

The ancient regulatory-protein family of WD-repeat proteins.

- 5 Neer EJ, Schmidt CJ, Nambudripad R, Smith TF;
Nature 1994;371:297-300.

Beta-transducin (G-beta) is one of the three subunits (alpha, beta, and gamma) of the guanine nucleotide-binding proteins (G proteins) which act as intermediaries in the transduction of signals generated by transmembrane
10 receptors [1]. The alpha subunit binds to and hydrolyzes GTP; the functions of the beta and gamma subunits are less clear but they seem to be required for the replacement of GDP by GTP as well as for membrane anchoring and receptor recognition.

- 15 In higher eukaryotes G-beta exists as a small multigene family of highly conserved proteins of about 340 amino acid residues. Structurally G-beta consists of eight tandem repeats of about 40 residues, each containing a central Trp-Asp motif (this type of repeat is sometimes called a WD-40 repeat). Such a repetitive segment has been shown [E1,2,3,4,5] to exist in a
20 number of other proteins listed below:

- Yeast STE4, a component of the pheromone response pathway. STE4 is a G-beta like protein that associates with GPA1 (G-alpha) and STE18 (G-gamma).
- Yeast MSI1, a negative regulator of RAS-mediated cAMP synthesis. MSI1 is
25 most probably also a G-beta protein.
- Human and chicken protein 12.3. The function of this protein is not known, but on the basis of its similarity to G-beta proteins, it may also function in signal transduction.
- 30 - Chlamydomonas reinhardtii gblp. This protein is most probably the homolog of vertebrate protein 12.3.
- Human LIS1, a neuronal protein involved in type-1 lissencephaly [E2].
- Mammalian coatamer beta' subunit (beta'-COP), a component of a cytosolic protein complex that reversibly associates with Golgi membranes to form

vesicles that mediate biosynthetic protein transport.

- Yeast CDC4, essential for initiation of DNA replication and separation of the spindle pole bodies to form the poles of the mitotic spindle.
- 5 - Yeast CDC20, a protein required for two microtubule-dependent processes: nuclear movements prior to anaphase and chromosome separation.
- Yeast MAK11, essential for cell growth and for the replication of M1 double-stranded RNA.
- Yeast PRP4, a component of the U4/U6 small nuclear ribonucleoprotein with
10 a probable role in mRNA splicing.
- Yeast PWP1, a protein of unknown function.
- Yeast SKI8, a protein essential for controlling the propagation of double-stranded RNA.
- Yeast SOF1, a protein required for ribosomal RNA processing which
15 associates with U3 small nucleolar RNA.
- Yeast TUP1 (also known as AER2 or SFL2 or CYC9), a protein which has been implicated in dTMP uptake, catabolite repression, mating sterility, and many other phenotypes.
- Yeast YCR57c, an ORF of unknown function from chromosome III.
- 20 - Yeast YCR72c, an ORF of unknown function from chromosome III.
- Slime mold coronin, an actin-binding protein.
- Slime mold AAC3, a developmentally regulated protein of unknown function.
- 25 - Drosophila protein Groucho (formerly known as E(spl); 'enhancer of split'), a protein involved in neurogenesis and that seems to interact with the Notch and Delta proteins.
- Drosophila TAF-II-80, a protein that is tightly associated with TFIID.
- 30 The number of repeats in the above proteins varies between 5 (PRP4, TUP1, and Groucho) and 8 (G-beta, STE4, MS11, AAC3, CDC4, PWP1, etc.). In G-beta and G-beta like proteins, the repeats span the entire length of the sequence, while in other proteins, they make up the N-terminal, the central or the C-terminal section.

A signature pattern can be developed from the central core of the domain (positions 9 to 23).

- 5 -Consensus pattern: [LIVMSTAC]-[LIVMFYWSTAGC]-[LIMSTAG]-[LIVMSTAGC]-x(2)-
[DN]-
x(2)-[LIVMWSTAC]-x-[LIVMFSTAG]-W-[DEN]-[LIVMFSTAGCN]

[1] Gilman A.G.

- 10 Annu. Rev. Biochem. 56:615-649(1987).

[2] Duronio R.J., Gordon J.I., Boguski M.S.
Proteins 13:41-56(1992).

[3] van der Voorn L., Ploegh H.L.
FEBS Lett. 307:131-134(1992).

- 15 [4] Neer E.J., Schmidt C.J., Nambudripad R., Smith T.F.
Nature 371:297-300(1994).

[5] Smith T.F., Gaiatzes C.G., Saxena K., Neer E.J.
Biochemistry In Press(1998).

20

718. WHEP-TRS domain containing proteins

A conserved domain of 46 amino acids has been shown [1] to exist in a number of higher eukaryote aminoacyl-transfer RNA synthetases. This domain is present one to six times in the following enzymes:

25

- Mammalian multifunctional aminoacyl-tRNA synthetase. The domain is present three times in a region that separates the N-terminal glutamyl-tRNA synthetase domain from the C-terminal prolyl-tRNA synthetase domain.

- Drosophila multifunctional aminoacyl-tRNA synthetase. The domain is present
30 six times in the intercatalytic region.

- Mammalian tryptophanyl-tRNA synthetase. The domain is found at the N-terminal extremity.

- Mammalian, insect, nematode and plant glycyl-tRNA synthetase. The domain is found at the N-terminal extremity [2].

- Mammalian histidyl-tRNA synthetase. The domain is found at the N-terminal extremity.

This domain, which is called WHEP-TRS, could contain a central alpha-helical region and may play a role in the association of tRNA-synthetases into multienzyme complexes.

A signature pattern based on the first 29 positions of the WHEP-Domain has been developed.

-Consensus pattern: [QY]-G-[DNEA]-x-[LIV]-[KR]-x(2)-K-x(2)-[KRNG]-[AS]-x(4)-[LIV]-[DENK]-x(2)-[IV]-x(2)-L-x(3)-K

[1] Cerini C., Kerjan P., Astier M., Gratecos D., Mirande M., Semeriva M.

EMBO J. 10:4267-4277(1991).

[2] Nada S., Chang P.K., Dignam J.D.

J. Biol. Chem. 268:7660-7667(1993).

719. (Worm family 8) Putative membrane protein

Analysis of protein domain families in *Caenorhabditis elegans*.

Sonnhammer EL, Durbin R;

Genomics 1997;46:200-216.

This family called family 8 in [1], may be a transmembrane protein

The specific function of this protein is unknown.

720. Xylose isomerase

Xylose isomerase (EC 5.3.1.5) [1] is an enzyme found in microorganisms which

catalyzes the interconversion of D-xylose to D-xylulose. It can also isomerize

D-ribose to D-ribulose and D-glucose to D-fructose. Xylose isomerase seems to

require magnesium for its activity, while cobalt is necessary to stabilize the

tetrameric structure of the enzyme. A number of residues are conserved in all

known xylose isomerases.

Xylose isomerase also exists in plants [2] where it is homodimeric and is manganese-dependent.

- 5 Two signatures patterns for xylose isomerase have been developed. The first one is derived from a stretch of five conserved amino acids that includes a glutamic acid residue known to be one of the four residues involved in the binding of the magnesium ion [3]; this pattern also includes a lysine residue which is involved in the catalytic activity. The second pattern is derived from a
- 10 conserved region in the N-terminal section of the enzyme that include an histidine residue which has been shown [4] to be involved in the catalytic mechanism of the enzyme.

-Consensus pattern: [LI]-E-P-K-P-x(2)-P

- 15 [E is a magnesium ligand]

[K is an active site residue]

-Consensus pattern: [FL]-H-D-x-D-[LIV]-x-[PD]-x-[GDE]

[H is an active site residue]

- 20 [1] Dauter Z., Dauter M., Hemker J., Witzel H., Wilson K.S.
FEBS Lett. 247:1-8(1989).

- [2] Kristo P.A., Saarelainen R., Fagerstrom R., Aho S., Korhola M.
Eur. J. Biochem. 237:240-246(1996).

- [3] Henrick K., Collyer C.A., Blow D.M.
25 J. Mol. Biol. 208:129-157(1989).

- [4] Vangrysperre W., Ampe C., Kersters-Hilderson H., Tempst P.
Biochem. J. 263:195-199(1989).

- 30 721. XPG protein signatures. Xeroderma pigmentosum (XP) [1] is a human autosomal recessive disease, characterized by a high incidence of sunlight-induced skin cancer. People's skin cells with this condition are hypersensitive to ultraviolet light, due to defects in the incision step of DNA excision repair. There are a minimum of seven genetic complementation groups involved in this pathway: XP-A to XP-G. The defect in XP-G can

be corrected by a 133 Kd nuclear protein called XPG (or XPGC) [2]. XPG belongs to a family of proteins [2,3,4,5,6] that are composed of two main subsets: - Subset 1, to which belongs XPG, RAD2 from budding yeast and rad13 from fission yeast. RAD2 and XPG are single-stranded DNA endonucleases [7,8]. XPG makes the 3' incision in human DNA nucleotide excision repair [9]. - Subset 2, to which belongs mouse and human FEN-1, rad2 from fission yeast, and RAD27 from budding yeast. FEN-1 is a structure-specific endonuclease. In addition to the proteins listed in the above groups, this family also includes: - Fission yeast exo1, a 5'→3' double-stranded DNA exonuclease that could act in a pathway that corrects mismatched base pairs. - Yeast EXO1 (DHS1), a protein with probably the same function as exo1. - Yeast DIN7. Sequence alignment of this family of proteins reveals that similarities are largely confined to two regions. The first is located at the N-terminal extremity (N-region) and corresponds to the first 95 to 105 amino acids. The second region is internal (I-region) and found towards the C-terminus; it spans about 140 residues and contains a highly conserved core of 27 amino acids that includes a conserved pentapeptide (E-A-[DE]-A-[QS]). It is possible that the conserved acidic residues are involved in the catalytic mechanism of DNA excision repair in XPG. The amino acids linking the N- and I-regions are not conserved; indeed, they are largely absent from proteins belonging to the second subset. Two signature patterns have been developed for these proteins. The first corresponds to the central part of the N-region, the second to part of the I-region and includes the putative catalytic core pentapeptide

Consensus pattern: [VI]-[KRE]-P-x-[FYIL]-V-F-D-G-x(2)-[PIL]-x-[LVC]-K-

Consensus pattern: [GS]-[LIVM]-[PER]-[FYS]-[LIVM]-x-A-P-x-E-A-[DE]-[PAS]-[QS]-[CLM]-

- [1] Tanaka K., Wood R.D. Trends Biochem. Sci. 19:83-86(1994).[2] Scherly D., Nospikel T., Corlet J., Ucla C., Bairoch A., Clarkson S.G. Nature 363:182-185(1993).[3] Carr A.M., Sheldrick K.S., Murray J.M., Al-Harithy R., Watts F.Z., Lehmann A.R. Nucleic Acids Res. 21:1345-1349(1993).[4] Murray J.M., Tavassoli M., Al-Harithy R., Sheldrick K.S., Lehmann A.R., Carr A.M., Watts F.Z. Mol. Cell. Biol. 14:4878-4888(1994).[5] Harrington J.J., Lieber M.R. Genes Dev. 8:1344-1355(1994).[6] Szankasi P., Smith G.R. Science 267:1166-1169(1995).[7] Habraken Y., Sung P., Prakash L., Prakash S. Nature 366:365-368(1993).[8] O'Donovan A., Scherly D., Clarkson S.G., Wood R.D. J. Biol. Chem.

269:15965-15968(1994).[9] O'Donovan A., Davies A.A., Moggs J.G., West S.C., Wood R.D. Nature 371:432-435(1994).

5 722. Xanthine/uracil permeases family

The following transport proteins which are involved in the uptake of xanthine or uracil are evolutionary related [1]:

- Uric acid-xanthine permease (gene uapA) from *Aspergillus nidulans*.
- 10 - Purine permease (gene uapC) from *Aspergillus nidulans*.
- Xanthine permease from *Bacillus subtilis* (gene pbuX).
- Uracil permease from *Escherichia coli* (gene uraA) [2] and *Bacillus* (gene pyrP).
- Hypothetical protein ycdG from *Escherichia coli*.
- 15 - Hypothetical protein ygfO from *Escherichia coli*.
- Hypothetical protein ygfU from *Escherichia coli*.
- Hypothetical protein yicE from *Escherichia coli*.
- Hypothetical protein yunJ from *Bacillus subtilis*.
- Hypothetical protein yunK from *Bacillus subtilis*.

20 They are proteins of from 430 to 595 residues that seem to contain 12 transmembrane domains.

The best conserved region which corresponds with what seems to be the tenth transmembrane domain has been selected as a signature pattern.

25 -Consensus pattern: [LIVM]-P-x-[PASIF]-V-[LIVM]-G-G-x(4)-[LIVM]-[FY]-[GSA]-x-[LIVM]-x(3)-G

[1] Diallinas G., Gorfinkiel L., Arst G., Cecchetto G., Scazzocchio C.
J. Biol. Chem. 270:8610-8622(1995).

30 [2] Andersen P.S., Frees D., Fast R., Mygind B.
J. Bacteriol. 177:2008-2013(1995).

723. Hypothetical yabO/yceC/sfhB family

The following proteins, which seems to belong to a family of pseudouridine synthases (EC 4.2.1.70) [1] have been shown to share regions of similarities:

- *Escherichia coli* and *Haemophilus influenzae* ribosomal large subunit pseudouridine synthase A (gene *rluA*). It is responsible for synthesis of pseudouridine from uracil-746 IN 23S rRNA.
- *Escherichia coli* and *Haemophilus influenzae* ribosomal large subunit pseudouridine synthase C (gene *rluC*). It is responsible for synthesis of pseudouridine from uracil at positions 955, 2504 and 2580 in 23S rRNA.
- *Escherichia coli* protein and homologs in other bacteria large subunit pseudouridine synthase D (gene *rluD*).
- Yeast DRAP deaminase (gene *RIB2*).
- *Escherichia coli* hypothetical protein *yqcB* and HI1435, the corresponding *Haemophilus influenzae* protein.
- *Haemophilus influenzae* hypothetical protein HI0042.
- *Aquifex aeolicus* hypothetical protein AQ_1758.
- *Bacillus subtilis* hypothetical protein *yhCT*.
- *Bacillus subtilis* hypothetical protein *yjbO*.
- *Bacillus subtilis* hypothetical protein *ylyB*.
- *Helicobacter pylori* hypothetical protein HP0347.
- *Helicobacter pylori* hypothetical protein HP0745.
- *Helicobacter pylori* hypothetical protein HP0956.
- *Mycoplasma genitalium* hypothetical protein MG209.
- *Mycoplasma genitalium* hypothetical protein MG370.
- *Synechocystis* strain PCC 6803 hypothetical protein *slr1592*.
- *Synechocystis* strain PCC 6803 hypothetical protein *slr1629*.
- Yeast hypothetical protein YDL036c.
- Yeast hypothetical protein YGR169c.
- Fission yeast hypothetical protein SpAC18B11.02c.
- *Caenorhabditis elegans* hypothetical protein K07E8.7.

These are proteins of from 21 to 50 Kd which contain a number of conserved regions in their central section. They can be picked up in the database by the following highly conserved pattern.

-Consensus pattern: [LIVCA]-[NHYT]-R-[LI]-D-x(2)-T-[STA]-G-[LIVAGC]-
[LIVMF](2)-[LIVMFGC]-[SGTACV]

- 5 [1] Conrad J., Sun D., Englund N., Ofengand J.
J. Biol. Chem. 273:18562-18566(1998).

In addition, the following bacterial proteins, which seems to belong to a family of
pseudouridine synthases (EC 4.2.1.70) [1] also have been shown to share regions of
10 similarities:

- Escherichia coli and Haemophilus influenzae 16S pseudouridylylate 516
synthase (EC 4.2.1.70) (gene: rsuA). This enzyme is responsible for the
formation of pseudouridine from uracil-516 in 16S ribosomal RNA.
- 15 - Escherichia coli hypothetical protein yciL and HI1199, the corresponding
Haemophilus influenzae protein.
- Escherichia coli hypothetical protein yjbC.
- Escherichia coli hypothetical protein ymfC and HI0694, the corresponding
Haemophilus influenzae protein.
- 20 - Aquifex aeolicus hypothetical protein AQ_554.
- Aquifex aeolicus hypothetical protein AQ_1464.
- Bacillus subtilis hypothetical protein ypuL.
- Bacillus subtilis hypothetical protein ytzF.
- Borrelia burgdorferi hypothetical protein BB0129.
- 25 - Helicobacter pylori hypothetical protein HP1459.
- Synechocystis strain PCC 6803 hypothetical protein slr0361.
- Synechocystis strain PCC 6803 hypothetical protein slr0612.

These are proteins of from 25 to 40 Kd which contain a number of conserved
30 regions in their central section. They can be picked up in the database by the
following highly conserved pattern.

-Consensus pattern: G-R-L-D-x(2)-[STA]-x-G-[LIVFA]-[LIVMF](3)-[ST]-[DNST]

[1] Wrzesinski J., Bakin A., Nurse K., Lane B.G., Ofengand J.
 Biochemistry 34:8904-8913(1995).

- 5 724. Zinc finger present in dystrophin, CBP/p300
 ZZ in dystrophin binds calmodulin
 Putative zinc finger; binding not yet shown.

10 725. Zinc carboxypeptidase

There are a number of different types of zinc-dependent carboxypeptidases (EC 3.4.17.-) [1,2]. All these enzymes seem to be structurally and functionally related. The enzymes that belong to this family are listed below.

- 15 - Carboxypeptidase A1 (EC 3.4.17.1), a pancreatic digestive enzyme that can remove all C-terminal amino acids with the exception of Arg, Lys and Pro.
 - Carboxypeptidase A2 (EC 3.4.17.15), a pancreatic digestive enzyme with a specificity similar to that of carboxypeptidase A1, but with a preference for bulkier C-terminal residues.
- 20 - Carboxypeptidase B (EC 3.4.17.2), also a pancreatic digestive enzyme, but that preferentially removes C-terminal Arg and Lys.
 - Carboxypeptidase N (EC 3.4.17.3) (also known as arginine carboxypeptidase), a plasma enzyme which protects the body from potent vasoactive and inflammatory peptides containing C-terminal Arg or Lys (such as kinins or anaphylatoxins) which are released into the circulation.
- 25 - Carboxypeptidase H (EC 3.4.17.10) (also known as enkephalin convertase or carboxypeptidase E), an enzyme located in secretory granules of pancreatic islets, adrenal gland, pituitary and brain. This enzyme removes residual C-terminal Arg or Lys remaining after initial endoprotease cleavage during
- 30 prohormone processing.
 - Carboxypeptidase M (EC 3.4.17.12), a membrane bound Arg and Lys specific enzyme.

It is ideally situated to act on peptide hormones at local tissue sites where it could control their activity before or after interaction with

specific plasma membrane receptors.

- Mast cell carboxypeptidase (EC 3.4.17.1), an enzyme with a specificity to carboxypeptidase A, but found in the secretory granules of mast cells.
- Streptomyces griseus carboxypeptidase (Cpase SG) (EC 3.4.17.-) [3], which combines the specificities of mammalian carboxypeptidases A and B.
- Thermoactinomyces vulgaris carboxypeptidase T (EC 3.4.17.18) (CPT) [4], which also combines the specificities of carboxypeptidases A and B.
- AEBP1 [5], a transcriptional repressor active in preadipocytes. AEBP1 seems to regulate transcription by cleavage of other transcriptional proteins.
- Yeast hypothetical protein YHR132c.

All of these enzymes bind an atom of zinc. Three conserved residues are implicated in the binding of the zinc atom: two histidines and a glutamic acid. Two signature patterns which contain these three zinc-ligands have been derived.

- Consensus pattern: [PK]-x-[LIVMFY]-x-[LIVMFY]-x(4)-H-[STAG]-x-E-x-[LIVM]-[STAG]-x(6)-[LIVMFYTA]
[H and E are zinc ligands]
- Consensus pattern: H-[STAG]-x(3)-[LIVME]-x(2)-[LIVMFYW]-P-[FYW]
[H is a zinc ligand]

[1] Tan F., Chan S.J., Steiner D.F., Schilling J.W., Skidgel R.A.
J. Biol. Chem. 264:13165-13170(1989).

[2] Reynolds D.S., Stevens R.L., Gurley D.S., Lane W.S., Austen K.F., Serafin W.E.
J. Biol. Chem. 264:20094-20099(1989).

[3] Narahashi Y.
J. Biochem. 107:879-886(1990).

[4] Teplyakov A., Polyakov K., Obmolova G., Strokopytov B., Kuranova I., Osterman A.L., Grishin N.V., Smulevitch S.V., Zagnitko O.P., Galperina O.V., Matz M.V., Stepanov V.M.
Eur. J. Biochem. 208:281-288(1992).

[5] He G.-P., Muise A., Li A.W., Ro H.-S.
Nature 378:92-96(1995).

[6] Hourdou M.-L., Guinand M., Vacheron M.J., Michel G., Denoroy L.,
Duez C.M., Englebert S., Joris B., Weber G., Ghuysen J.-M.
Biochem. J. 292:563-570(1993).

[7] Rawlings N.D., Barrett A.J.

5 Meth. Enzymol. 248:183-228(1995).

726. Zinc finger, C2H2 type

The C2H2 zinc finger is the classical zinc finger domain.

10 The two conserved cysteines and histidines co-ordinate a
zinc ion. The following pattern describes the zinc finger.

#-X-C-X(1-5)-C-X3-#-X5-#-X2-H-X(3-6)-[H/C]

Where X can be any amino acid, and numbers in brackets
indicate the number of residues. The positions marked # are

15 those that are important for the stable fold of the zinc
finger. The final position can be either his or cys.

The C2H2 zinc finger is composed of two short beta strands
followed by an alpha helix. The amino terminal part of the
helix binds the major groove in DNA binding zinc fingers.

20 'Zinc finger' domains [1-5] are nucleic acid-binding protein structures first
identified in the *Xenopus* transcription factor TFIIIA. These domains have
since been found in numerous nucleic acid-binding proteins. A zinc finger
domain is composed of 25 to 30 amino-acid residues. There are two cysteine or
25 histidine residues at both extremities of the domain, which are involved in
the tetrahedral coordination of a zinc atom. It has been proposed that such a
domain interacts with about five nucleotides. A schematic representation of a
zinc finger domain is shown below:

30 x x
x x
x x
x x
x x

x x
 C H
 x \ / x
 x Zn x
 5 x / \ x
 C H
 x x x x x x x x x x

Many classes of zinc fingers are characterized according to the number and positions of the histidine and cysteine residues involved in the zinc atom coordination. In the first class to be characterized, called C2H2, the first pair of zinc coordinating residues are cysteines, while the second pair are histidines. A number of experimental reports have demonstrated the zinc-dependent DNA or RNA binding property of some members of this class.

Some of the proteins known to include C2H2-type zinc fingers are listed below. The number of zinc finger regions found in each of these proteins are indicated between brackets; a '+' symbol indicates that only partial sequence data is available and that additional finger domains may be present.

- *Saccharomyces cerevisiae*: ACE2 (3), ADR1 (2), AZF1 (4), FZF1 (5), MIG1 (2), MSN2 (2), MSN4 (2), RGM1 (2), RIM1 (3), RME1 (3), SFP1 (2), SSL1 (1), STP1 (3), SWI5 (3), VAC1 (1) and ZMS1 (2).
- *Emericella nidulans*: brlA (2), creA (2).
- *Drosophila*: AEF-1 (4), Cf2 (7), ci-D (5), Disconnected (2), Escargot (5), Glass (5), Hunchback (6), Kruppel (5), Kruppel-H (4+), Odd-skipped (4), Odd-paired (4), Pep (3), Snail (5), Spalt-major (7), Serependity locus beta (6), delta (7), h-1 (8), Suppressor of hairy wing su(Hw) (12), Suppressor of variegation suvar(3)7 (5), Teashirt (3) and Tramtrack (2).
- *Xenopus*: transcription factor TFIIIA (9), p43 from RNP particle (9), Xfin (37 !!), Xsna (5), gastrula XlclGF5.1 to XlclGF71.1 (from 4+ to 11+), Oocyte XlclOF2 to XlclOF22 (from 7 to 12).
- Mammalian: basonuclin (6), BCL-6/LAZ-3 (6), erythroid krueppel-like transcription factor (3), transcription factors Sp1 (3), Sp2 (3), Sp3 (3)

and Sp(4) 3, transcriptional repressor YY1 (4), Wilms' tumor protein (4), EGR1/Krox24 (3), EGR2/Krox20 (3), EGR3/Pilot (3), EGR4/AT133 (4), Evi-1 (10), GLI1 (5), GLI2 (4+), GLI3 (3+), HIV-EP1/ZNF40 (4), HIV-EP2 (2), KR1 (9+), KR2 (9), KR3 (15+), KR4 (14+), KR5 (11+), HF.12 (6+), REX-1 (4), ZfX (13), ZfY (13), Zfp-35 (18), ZNF7 (15), ZNF8 (7), ZNF35 (10), ZNF42/MZF-1 (13), ZNF43 (22), ZNF46/Kup (2), ZNF76 (7), ZNF91 (36), ZNF133 (3).

In addition to the conserved zinc ligand residues it has been shown [6] that a number of other positions are also important for the structural integrity of the C2H2 zinc fingers. The best conserved position is found four residues after the second cysteine; it is generally an aromatic or aliphatic residue.

-Consensus pattern: C-x(2,4)-C-x(3)-[LIVMFYWC]-x(8)-H-x(3,5)-H
[The two C's and two H's are zinc ligands]

[1] Klug A., Rhodes D.

Trends Biochem. Sci. 12:464-469(1987).

[2] Evans R.M., Hollenberg S.M.

Cell 52:1-3(1988).

[3] Payre F., Vincent A.

FEBS Lett. 234:245-250(1988).

[4] Miller J., McLachlan A.D., Klug A.

EMBO J. 4:1609-1614(1985).

[5] Berg J.M.

Proc. Natl. Acad. Sci. U.S.A. 85:99-102(1988).

[6] Rosenfeld R., Margalit H.

J. Biomol. Struct. Dyn. 11:557-570(1993).

727. Zinc finger, C3HC4 type (RING finger)

A number of eukaryotic and viral proteins contain a conserved cysteine-rich domain of 40 to 60 residues (called C3HC4 zinc-finger or 'RING' finger) [1] that binds two atoms of zinc, and is probably involved in mediating protein-protein interactions. The 3D structure of the zinc ligation system is unique

to the RING domain and is referred to as the "cross-brace" motif. The spacing of the cysteines in such a domain is C-x(2)-C-x(9 to 39)-C-x(1 to 3)-H-x(2 to 3)-C-x(2)-C-x(4 to 48)-C-x(2)-C.

- 5 Proteins currently known to include the C3HC4 domain are listed below (references are only provided for recently determined sequences).

- Mammalian V(D)J recombination activating protein (gene RAG1). RAG1 activates the rearrangement of immunoglobulin and T-cell receptor genes.
- 10 - Mouse rpt-1. Rpt-1 is a trans-acting factor that regulates gene expression directed by the promoter region of the interleukin-2 receptor alpha chain or the LTR promoter region of HIV-1.
- Human rfp. Rfp is a developmentally regulated protein that may function in male germ cell development. Recombination of the N-terminal section of rfp
- 15 with a protein tyrosine kinase produces the ret transforming protein.
- Human 52 Kd Ro/SS-A protein. A protein of unknown function from the Ro/SS-A ribonucleoprotein complex. Sera from patients with systemic lupus erythematosus or primary Sjogren's syndrome often contain antibodies that react with the Ro proteins.
- 20 - Human histocompatibility locus protein RING1.
- Human PML, a probable transcription factor. Chromosomal translocation of PML with retinoic receptor alpha creates a fusion protein which is the cause of acute promyelocytic leukemia (APL).
- Mammalian breast cancer type 1 susceptibility protein (BRCA1) [E1].
- 25 - Mammalian cbl proto-oncogene.
- Mammalian bmi-1 proto-oncogene.
- Vertebrate CDK-activating kinase (CAK) assembly factor MAT1, a protein that stabilizes the complex between the CDK7 kinase and cyclin H (MAT1 stands for 'Menage A Trois').
- 30 - Mammalian mel-18 protein. Mel-18 which is expressed in a variety of tumor cells is a transcriptional repressor that recognizes and binds a specific DNA sequence.
- Mammalian peroxisome assembly factor-1 (PAF-1) (PMP35), which is somewhat involved in the biogenesis of peroxisomes. In humans, defects in PAF-1 are

responsible for a form of Zellweger syndrome, an autosomal recessive disorder associated with peroxisomal deficiencies.

- Human MAT1 protein, which interacts with the CDK7-cyclin H complex.
- Human RING1 protein.
- 5 - Xenopus XNF7 protein, a probable transcription factor.
- Trypanosoma protein ESAG-8 (T-LR), which may be involved in the posttranscriptional regulation of genes in VSG expression sites or may interact with adenylate cyclase to regulate its activity.
- Drosophila proteins Posterior Sex Combs (Psc) and Suppressor two of zeste (Su(z)2). The two proteins belong to the Polycomb group of genes needed to maintain the segment-specific repression of homeotic selector genes.
- 10 - Drosophila protein male-specific msl-2, a DNA-binding protein which is involved in X chromosome dosage compensation (the elevation of transcription of the male single X chromosome).
- 15 - Arabidopsis thaliana protein COP1 which is involved in the regulation of photomorphogenesis.
- Fungal DNA repair proteins RAD5, RAD16, RAD18 and rad8.
- Herpesviruses trans-acting transcriptional protein ICP0/IE110. This protein which has been characterized in many different herpesviruses is a trans-activator and/or -repressor of the expression of many viral and cellular promoters.
- 20 - Baculoviruses protein CG30.
- Baculoviruses major immediate early protein (PE-38).
- Baculoviruses immediate-early regulatory protein IE-N/IE-2.
- 25 - Caenorhabditis elegans hypothetical proteins F54G8.4, R05D3.4 and T02C1.1.
- Yeast hypothetical proteins YER116c and YKR017c.

The central region of the domain was selected as a signature pattern for the C3HC4 finger.

-Consensus pattern: C-x-H-x-[LIVMFY]-C-x(2)-C-[LIVMYA]

[1] Borden K.L.B., Freemont P.S.

Curr. Opin. Struct. Biol. 6:395-401(1996).

728. Zinc finger C-x8-C-x5-C-x3-H type (and similar).

5

729. Zinc finger, CCHC class

A family of CCHC zinc fingers, mostly from retroviral gag proteins (nucleocapsid). Prototype structure is from HIV.

Also contains members involved in eukaryotic gene

10 regulation, such as *C. elegans* GLH-1.

Structure is an 18-residue zinc finger; no examples of indels in the alignment.

15 730. Zn-finger in Ran binding protein and others.

731. AN1-like Zinc finger

20 Zinc finger at the C-terminus of An1 [Swiss:Q91839](#), a ubiquitin-like protein in *Xenopus laevis*. The following pattern describes the zinc finger. C-X2-C-X(9-12)-C-X(1-2)-C-X4-C-X2-H-X5-H-X-C Where X can be any amino acid, and numbers in brackets indicate the number of residues.

25 [1] Linnen JM, Bailey CP, Weeks DL; Gene 1993;128:181-188.

732. 14-3-3 proteins

Structure of a 14-3-3 protein and implications for coordination of multiple signalling pathways.

30

Xiao B, Smerdon SJ, Jones DH, Dodson GG, Soneji Y, Aitken A, Gamblin SJ; Nature 1995;376:188-191.

Crystal structure of the zeta isoform of the 14-3-3 protein.

Liu D, Bienkowska J, Petosa C, Collier RJ, Fu H, Liddington R;

Nature 1995;376:191-194.

Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphoserine.

- 5 Muslin AJ, Tanner JW, Allen PM, Shaw AS;
Cell 1996;84:889-897.

The 14-3-3 protein binds its target proteins with a common site located towards the C-terminus.

- 10 Ichimura T, Ito M, Itagaki C, Takahashi M, Horigome T, Omata S, Ohno S, Isobe T
FEBS Lett 1997;413:273-276.

Molecular evolution of the 14-3-3 protein family.

- 15 Wang W, Shakes DC
J Mol Evol 1996;43:384-398.
Function of 14-3-3 proteins.
Jin DY, Lyu MS, Kozak CA, Jeang KT
Nature 1996;382:308-308.

- 20 The 14-3-3 proteins [1,2,3] are a family of closely related acidic homodimeric proteins of about 30 Kd which were first identified as being very abundant in mammalian brain tissues and located preferentially in neurons. The 14-3-3 proteins seem to have multiple biological activities and play a key role in
25 signal transduction pathways and the cell cycle. They interacts with kinases such as PKC or Raf-1; they seem to also function as protein-kinase dependent activators of tyrosine and tryptophan hydroxylases and in plants they are associated with a complex that binds to the G-box promoter elements.

- 30 The 14-3-3 family of proteins are ubiquitously found in all eukaryotic species studied and have been sequenced in fungi (yeast BMH1 and BMH2, fission yeast rad24 and rad25), plants, Drosophila, and vertebrates. The sequences of the 14-3-3 proteins are extremely well conserved. Two highly conserved regions have been selected as signature patterns: the first is a peptide of 11 residues

located in the N-terminal section; the second, a 20 amino acid region located in the C-terminal section.

-Consensus pattern: R-N-L-[LIV]-S-[VG]-[GA]-Y-[KN]-N-[IVA]

5 -Consensus pattern: Y-K-[DE]-S-T-L-I-[IM]-Q-L-[LF]-[RHC]-D-N-[LF]-T-[LS]-W-[TAN]-[SAD]

[1] Aitken A.

Trends Biochem. Sci. 20:95-97(1995).

10 [2] Morrison D.

Science 266:56-57(1994).

[3] Xiao B., Smerdon S.J., Jones D.H., Dodson G.G., Soneji Y., Aitken A., Gamblin S.J.

Nature 376:188-191(1995).

733. D-isomer specific 2-hydroxyacid dehydrogenases (2 Hacid DH)

This Pfam covers the Formate dehydrogenase, D-glycerate dehydrogenase and D-lactate dehydrogenase families in SCOP. A number of NAD-dependent 2-hydroxyacid dehydrogenases which seem to be specific for the D-isomer of their substrate have been shown [1,2,3,4] to be functionally and structurally related. These enzymes are listed below.

- D-lactate dehydrogenase (EC 1.1.1.28), a bacterial enzyme which catalyzes the reduction of D-lactate to pyruvate.
- D-glycerate dehydrogenase (EC 1.1.1.29) (NADH-dependent hydroxypyruvate reductase), a plant leaf peroxisomal enzyme that catalyzes the reduction of hydroxypyruvate to glycerate. This reaction is part of the glycolate pathway of photorespiration.
- D-glycerate dehydrogenase from the bacteria *Hyphomicrobium methylovorum* and *Methylobacterium extorquens*.
- 3-phosphoglycerate dehydrogenase (EC 1.1.1.95), a bacterial enzyme that catalyzes the oxidation of D-3-phosphoglycerate to 3-phosphohydroxypyruvate. This reaction is the first committed step in the 'phosphorylated' pathway of serine biosynthesis.

- Erythronate-4-phosphate dehydrogenase (EC 1.1.1.-) (gene *pdxB*), a bacterial enzyme involved in the biosynthesis of pyridoxine (vitamin B6).
- D-2-hydroxyisocaproate dehydrogenase (EC 1.1.1.-) (*D*-*hicDH*), a bacterial enzyme that catalyzes the reversible and stereospecific interconversion between 2-ketocarboxylic acids and D-2-hydroxy-carboxylic acids.
- Formate dehydrogenase (EC 1.2.1.2) (FDH) from the bacteria *Pseudomonas* sp. 101 and various fungi [5].
- Vancomycin resistance protein *vanH* from *Enterococcus faecium*; this protein is a D-specific alpha-keto acid dehydrogenase involved in the formation of a peptidoglycan which does not terminate by D-alanine thus preventing vancomycin binding.
- *Escherichia coli* hypothetical protein *ycdW*.
- *Escherichia coli* hypothetical protein *yiaE*.
- *Haemophilus influenzae* hypothetical protein *HI1556*.
- Yeast hypothetical protein *YER081w*.
- Yeast hypothetical protein *YIL074w*.

All these enzymes have similar enzymatic activities and are structurally related. Three of the most conserved regions of these proteins have been selected to develop patterns. The first pattern is based on a glycine-rich region located in the central section of these enzymes; this region probably corresponds to the NAD-binding domain. The two other patterns contain a number of conserved charged residues, some of which may play a role in the catalytic mechanism.

-Consensus pattern: [LIVMA]-[AG]-[IVT]-[LIVMFY]-[AG]-x-G-[NHKRQGSAC]-[LIV]-

G-x(13,14)-[LIVfMT]-x(2)-[FYwCTH]-[DNSTK]

-Consensus pattern: [LIVMFYWA]-[LIVFYWC]-x(2)-[SAC]-[DNQHR]-[IVFA]-[LIVF]-x-[LIVF]-[HNI]-x-P-x(4)-[STN]-x(2)-[LIVMF]-x-[GSDN]

-Consensus pattern: [LMFATC]-[KPQ]-x-[GSTDN]-x-[LIVMFYWR]-[LIVMFYW](2)-N-x-[STAGC]-R-[GP]-x-[LIVH]-[LIVMC]-[DNV]

[1] Grant G.A. *Biochem. Biophys. Res. Commun.* 165:1371-1374(1989).

[2] Kochhar S., Hunziker P., Leong-Morgenthaler P.M., Hottinger H. *Biochem. Biophys. Res. Commun.* 184:60-66(1992).

[3] Ohta T., Taguchi H. *J. Biol. Chem.* 266:12588-12594(1991).

[4] Goldberg J.D., Yoshida T., Brick P. J. Mol. Biol. 236:1123-1140(1994).

[5] Popov V.O., Lamzin V.S. Biochem. J. 301:625-643(1994).

- 5 734. 2-oxo acid dehydrogenases acyltransferase (catalytic domain)
Refined crystal structure of the catalytic domain of dihydrolipoyl
transacetylase (E2P) from azotobacter vineelandii at 2.6 angstroms
resolution.

Mattevi A, Obmolova G, Kalk KH, Westphal AH, De Kok A, Hol WG;

- 10 J Mol Biol 1993;230:1183-1199.

These proteins contain one to three copies of a lipoyl binding domain
followed by the catalytic domain.

- 15 735. 3-beta hydroxysteroid dehydrogenase/isomerase family
Structure and tissue-specific expression of 3
beta-hydroxysteroid dehydrogenase/5-ene-4-ene isomerase
genes in human and rat classical and peripheral
steroidogenic tissues.

- 20 Labrie F, Simard J, Luu-The V, Pelletier G, Belanger A,
Lachance Y, Zhao HF, Labrie C, Breton N, de Launoit Y, et al
J Steroid Biochem Mol Biol 1992;41:421-435.

The enzyme 3 beta-hydroxysteroid dehydrogenase/5-ene-4-ene
isomerase (3 beta-HSD) catalyzes the oxidation and isomerization

- 25 of 5-ene-3 beta-hydroxypregnene and 5-ene-hydroxyandrostene
steroid precursors into the corresponding 4-ene-ketosteroids necessary
for the formation of all classes of steroid hormones.

- 30 736. 3-hydroxyacyl-CoA dehydrogenase

This family also includes lambda crystallin.

Structure of L-3-hydroxyacyl-coenzyme A dehydrogenase:
preliminary chain tracing at 2.8-A resolution.

Birktoft JJ, Holden HM, Hamlin R, Xuong NH, Banaszak LJ;

Proc Natl Acad Sci U S A 1987;84:8262-8266.

3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) (HCDH) [1] is an enzyme involved in fatty acid metabolism, it catalyzes the reduction of 3-hydroxyacyl-CoA to 3-oxoacyl-CoA. Most eukaryotic cells have 2 fatty-acid beta-oxidation systems, one located in mitochondria and the other in peroxisomes. In peroxisomes 3-hydroxyacyl-CoA dehydrogenase forms, with enoyl-CoA hydratase (ECH) and 3,2-trans-enoyl-CoA isomerase (ECI) a multifunctional enzyme where the N-terminal domain bears the hydratase/isomerase activities and the C-terminal domain the dehydrogenase activity. There are two mitochondrial enzymes: one which is monofunctional and the other which is, like its peroxisomal counterpart, multifunctional.

In *Escherichia coli* (gene *fadB*) and *Pseudomonas fragi* (gene *faoA*) HCDH is part of a multifunctional enzyme which also contains an ECH/ECI domain as well as a 3-hydroxybutyryl-CoA epimerase domain [2].

The other proteins structurally related to HCDH are:

- Bacterial 3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157) which reduces 3-hydroxybutanoyl-CoA to acetoacetyl-CoA [3].
- Eye lens protein lambda-crystallin [4], which is specific to lagomorphes (such as rabbit).

There are two major region of similarities in the sequences of proteins of the HCDH family, the first one located in the N-terminal, corresponds to the NAD-binding site, the second one is located in the center of the sequence. A signature pattern has been derived from this central region.

-Consensus pattern: [DNE]-x(2)-[GA]-F-[LIVMFY]-x-[NT]-R-x(3)-[PA]-[LIVMFY](2)-x(5)-[LIVMFYCT]-[LIVMFY]-x(2)-[GV]

[1] Birktoff J.J., Holden H.M., Hamlin R., Xuong N.-H., Banaszak L.J.

Proc. Natl. Acad. Sci. U.S.A. 84:8262-8266(1987).

[2] Nakahigashi K., Inokuchi H.

Nucleic Acids Res. 18:4937-4937(1990).

[3] Mullany P., Clayton C.L., Pallen M.J., Slone R., Al-Saleh A.,
Tabaqchali S.

5 FEMS Microbiol. Lett. 124:61-67(1994).

[4] Mulders J.W.M., Hendriks W., Blankesteyn W.M., Bloemendal H.,
de Jong W.W.
J. Biol. Chem. 263:15462-15466(1988).

10

737. 60s Acidic ribosomal protein

Proteins P1, P2, and P0, components of the eukaryotic
ribosome stalk. New structural and functional aspects.

Remacha M, Jimenez-Diaz A, Santos C, Briones E, Zambrano R,

15 Rodriguez Gabriel MA, Guarinos E, Ballesta JP;

Biochem Cell Biol 1995;73:959-968.

This family includes archaebacterial L12, eukaryotic P0, P1 and P2.

20 738. 6-phosphogluconate dehydrogenases

6-phosphogluconate dehydrogenase (EC 1.1.1.44) (6PGD) catalyzes the third step
in the hexose monophosphate shunt, the decarboxylating reduction of
6-phosphogluconate in to ribulose 5-phosphate.

25 Prokaryotic and eukaryotic 6PGD are proteins of about 470 amino acids whose
sequence are highly conserved [1]. A region which has been shown [2], from studies
of the sheep 6PGD tertiary structure, to be involved in the binding of 6-phosphogluconate
has been selected as a signature pattern.

30 -Consensus pattern: [LIVM]-x-D-x(2)-[GA]-[NQS]-K-G-T-G-x-W

[1] Reizer A., Deutscher J., Saier M.H. Jr., Reizer J.
Mol. Microbiol. 5:1081-1089(1991).

[2] Adams M.J., Archibald I.G., Bugg C.E., Carne A., Gover S.,

Helliwell J.R., Pickersgill R.W., White S.W.

EMBO J. 2:1009-1014(1983).

5 739. (7m 1) G-protein coupled receptors [1 to 4,E1,E2] (also called R7G) are an extensive group of hormones, neurotransmitters, odorants and light receptors which transduce extracellular signals by interaction with guanine nucleotide-binding (G) proteins. The receptors that are currently known to belong to this family are listed below.

10

- 5-hydroxytryptamine (serotonin) 1A to 1F, 2A to 2C, 4, 5A, 5B, 6 and 7 [5].
- Acetylcholine, muscarinic-type, M1 to M5.
- Adenosine A1, A2A, A2B and A3 [6].
- Adrenergic alpha-1A to -1C; alpha-2A to -2D; beta-1 to -3 [7].

15

- Angiotensin II types I and II.
- Bombesin subtypes 3 and 4.
- Bradykinin B1 and B2.
- c3a and C5a anaphylatoxin.
- Cannabinoid CB1 and CB2.

20

- Chemokines C-C CC-CKR-1 to CC-CKR-8.
- Chemokines C-X-C CXC-CKR-1 to CXC-CKR-4.
- Cholecystokinin-A and cholecystokinin-B/gastrin.
- Dopamine D1 to D5 [8].
- Endothelin ET-a and ET-b [9].

25

- fMet-Leu-Phe (fMLP) (N-formyl peptide).
- Follicle stimulating hormone (FSH-R) [10].
- Galanin.
- Gastrin-releasing peptide (GRP-R).
- Gonadotropin-releasing hormone (GNRH-R).

30

- Histamine H1 and H2 (gastric receptor I).
- Lutropin-choriogonadotropic hormone (LSH-R) [10].
- Melanocortin MC1R to MC5R.
- Melatonin.
- Neuromedin B (NMB-R).

- Neuromedin K (NK-3R).
- Neuropeptide Y types 1 to 6.
- Neurotensin (NT-R).
- Octopamine (tyramine), from insects.
- 5 - Odorants [11].
- Opioids delta-, kappa- and mu-types [12].
- Oxytocin (OT-R).
- Platelet activating factor (PAF-R).
- Prostacyclin.
- 10 - Prostaglandin D2.
- Prostaglandin E2, EP1 to EP4 subtypes.
- Prostaglandin F2.
- Purinoreceptors (ATP) [13].
- Somatostatin types 1 to 5.
- 15 - Substance-K (NK-2R).
- Substance-P (NK-1R).
- Thrombin.
- Thromboxane A2.
- Thyrotropin (TSH-R) [10].
- 20 - Thyrotropin releasing factor (TRH-R).
- Vasopressin V1a, V1b and V2.
- Visual pigments (opsins and rhodopsin) [14].
- Proto-oncogene mas.
- A number of orphan receptors (whose ligand is not known) from mammals and
- 25 birds.
- Caenorhabditis elegans putative receptors C06G4.5, C38C10.1, C43C3.2, T27D1.3 and ZC84.4.
- Three putative receptors encoded in the genome of cytomegalovirus: US27, US28, and UL33.
- 30 - ECRF3, a putative receptor encoded in the genome of herpesvirus saimiri.

The structure of all these receptors is thought to be identical. They have seven hydrophobic regions, each of which most probably spans the membrane. The N-terminus is located on the extracellular side of the membrane and is

often glycosylated, while the C-terminus is cytoplasmic and generally phosphorylated. Three extracellular loops alternate with three intracellular loops to link the seven transmembrane regions. Most, but not all of these receptors, lack a signal peptide. The most conserved parts of these proteins are the transmembrane regions and the first two cytoplasmic loops. A conserved acidic-Arg-aromatic triplet is present in the N-terminal extremity of the second cytoplasmic loop [15] and could be implicated in the interaction with G proteins.

To detect this widespread family of proteins, a pattern that contains the conserved triplet and that also spans the major part of the third transmembrane helix has been developed.

-Consensus pattern: [GSTALIVMFYWC]-[GSTANCPDE]-{EDPKRH}-x(2)-
[LIVMNQGA]-x(2)-
[LIVMFT]-[GSTANC]-[LIVMFYWSTAC]-[DENH]-R-[FYWCSH]-x(2)-
[LIVM]

[1] Strosberg A.D.

Eur. J. Biochem. 196:1-10(1991).

[2] Kerlavage A.R.

Curr. Opin. Struct. Biol. 1:394-401(1991).

[3] Probst W.C., Snyder L.A., Schuster D.I., Brosius J., Sealfon S.C.

DNA Cell Biol. 11:1-20(1992).

[4] Savarese T.M., Fraser C.M.

Biochem. J. 283:1-9(1992).

[5] Branchek T.

Curr. Biol. 3:315-317(1993).

[6] Stiles G.L.

J. Biol. Chem. 267:6451-6454(1992).

[7] Friell T., Kobilka B.K., Lefkowitz R.J., Caron M.G.

Trends Neurosci. 11:321-324(1988).

[8] Stevens C.F.

Curr. Biol. 1:20-22(1991).

[9] Sakurai T., Yanagisawa M., Masaki T.

Trends Pharmacol. Sci. 13:103-107(1992).

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Biochimie 73:109-120(1991).

5 [11] Lancet D., Ben-Arie N.

Curr. Biol. 3:668-674(1993).

[12] Uhl G.R., Childers S., Pasternak G.

Trends Neurosci. 17:89-93(1994).

[13] Barnard E.A., Burnstock G., Webb T.E.

10 Trends Pharmacol. Sci. 15:67-70(1994).

[14] Applebury M.L., Hargrave P.A.

Vision Res. 26:1881-1895(1986).

[15] Attwood T.K., Eliopoulos E.E., Findlay J.B.C.

Gene 98:153-159(1991).

15 (7tm 1) Visual pigments (opsins) retinal binding site

Visual pigments [1,2] are the light-absorbing molecules that mediate vision.

They consist of an apoprotein, opsin, covalently linked to the chromophore cis-retinal. Vision is effected through the absorption of a photon by cis-

20 retinal which is isomerized to trans-retinal. This isomerization leads to a

change of conformation of the protein. Opsins are integral membrane proteins with seven transmembrane regions that belong to family 1 of G-protein coupled receptors.

25 In vertebrates four different pigments are generally found. Rod cells, which mediate vision in dim light, contain the pigment rhodopsin. Cone cells, which function in bright light, are responsible for color vision and contain three or more color pigments (for example, in mammals: red, blue and green).

30 In Drosophila, the eye is composed of 800 facets or ommatidia. Each ommatidium contains eight photoreceptor cells (R1-R8): the R1 to R6 cells are outer cells, R7 and R8 inner cells. Each of the three types of cells (R1-R6, R7 and R8) expresses a specific opsin.

Proteins evolutionary related to opsins include squid retinochrome, also known as retinal photoisomerase, which converts various isomers of retinal into 11-cis retinal and mammalian retinal pigment epithelium (RPE) RGR [3], a protein that may also act in retinal isomerization.

5

The attachment site for retinal in the above proteins is a conserved lysine residue in the middle of the seventh transmembrane helix. The pattern that had been developed includes this residue.

10 -Consensus pattern: [LIVMWAC]-[PGC]-x(3)-[SAC]-K-[STALIMR]-[GSACPNV]-[STACP]-

x(2)-[DENF]-[AP]-x(2)-[LY]

[K is the retinal binding site]

15 [1] Applebury M.L., Hargrave P.A.

Vision Res. 26:1881-1895(1986).

[2] Fryxell K.J., Meyerowitz E.M.

J. Mol. Evol. 33:367-378(1991).

[3] Shen D., Jiang M., Hao W., Tao L., Salazar M., Fong H.K.W.

20 Biochemistry 33:13117-13125(1994).

The following descriptions of protein family functions are not provided by the Pfam or Prosite databases.

25

740. BAH

BAH domain. Number of members: 65

30 [1] Medline: 97074677. Molecular cloning of polybromo, a nuclear protein containing multiple domains including five bromodomains, a truncated HMG-box, and two repeats of a novel domain. Nicolas RH, Goodwin GH; Gene 1996;175:233-240.

[2] Medline: 99198739. The BAH (bromo-adjacent homology) domain: a link between DNA methylation, replication and transcriptional regulation. Callebaut I, Courvalin J-C, Mornon JP; FEBS letts 1999;446:189-193.

741. ELM2.

ELM2 domain. The ELM2 (Egl-27 and MTA1 homology 2) domain is a small domain of

5 unknown function. Number of members: 10

742. Euk proin. EUKARYOTIC_PORIN The major protein of the outer mitochondrial membrane of eukaryotes is a porin that forms a voltage-dependent anion-selective
10 channel (VDAC) that behaves as a general diffusion pore for small hydrophilic molecules [1 to 4]. The channel adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV.

This protein contains about 280 amino acids and its sequence is composed of between 12
15 to 16 beta-strands that span the mitochondrial outer membrane. Yeast contains two members of this family (genes POR1 and POR2); vertebrates have at least three members (genes VDAC1, VDAC2 and VDAC3) [5].

A conserved region located at the C-terminal part of these proteins was selected as a signature pattern.

20 Consensus pattern[YH]-x(2)-D-[SPCAD]-x-[STA]-x(3)-[TAG]-[KR]-[LIVMF]-[DNSTA]-[DNS]-x(4)-[GSTAN]-[LIVMA]-x-[LIVMY]

[1] Benz R. Biochim. Biophys. Acta 1197:167-196(1994).

[2] Manella C.A. Trends Biochem. Sci. 17:315-320(1992).

25 [3] Dihanich M. Experientia 46:146-153(1990).

[4] Forte M., Guy H.R., Mannella C.A. J. Bioenerg. Biomembr. 19:341-350(1987).

[5] Sampson M.J., Lovell R.S., Davison D.B., Craigen W.J. Genomics 36:192-196(1996).

30 743. Glyco hydor 19

Chitinases family 19 signatures

cross-reference(s) CHITINASE_19_1, CHITINASE_19_2

Chitinases (EC 3.2.1.14) [1] are enzymes that catalyze the hydrolysis of the beta-1,4-N-acetyl-D-glucosamine linkages in chitin polymers. From the view point of sequence

similarity chitinases belong to either family 18 or 19 in the classification of glycosyl hydrolases [2,E1]. Chitinases of family 19 (also known as classes IA or I and IB or II) are enzymes from plants that function in the defense against fungal and insect pathogens by destroying their chitin-containing cell wall. Class IA/I and IB/II enzymes differ in the presence (IA/I) or absence (IB/II) of a N-terminal chitin-binding domain (see the relevant entry <PDOC00025>). The catalytic domain of these enzymes consist of about 220 to 230 amino acid residues.

Two highly conserved regions were selected as signature patterns, the first one is located in the N-terminal section and contains one of the six cysteines which are conserved in most, if not all, of these chitinases and which is probably involved in a disulfide bond.

Consensus pattern C-x(4,5)-F-Y-[ST]-x(3)-[FY]-[LIVMF]-x-A-x(3)-[YF]-x(2)-F-[GSA]
Consensus pattern [LIVM]-[GSA]-F-x-[STAG](2)-[LIVMFY]-W-[FY]-W-[LIVM]

[1]Flach J., Pilet P.-E., Jolles P. *Experientia* 48:701-716(1992).

[2] Henrissat B. *Biochem. J.* 280:309-316(1991).

744. MBD

Methyl-CpG binding domain

The Methyl-CpG binding domain (MBD) binds to DNA that contains one or more symmetrically methylated CpGs [1]. DNA methylation in animals is associated with alterations in chromatin structure and silencing of gene expression. MBD has negligible non-specific affinity for DNA. In vitro foot-printing with MeCP2 showed the MBD can protect a 12 nucleotide region surrounding a methyl CpG pair [1]. MBDs are found in several Methyl-CpG binding proteins and also DNA demethylase [2]. Number of members: 11

[1]Medline: 94232813. Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2. Nan X, Meehan RR, Bird A; *Nucleic Acids Res* 1993;21:4886-4892.

[2]Medline: 99158138. A mammalian protein with specific demethylase activity for mCpG DNA. Bhattacharya SK, Ramchandani S, Cervoni N, Szyf M; *Nature* 1999;397:579-583.

745. Peptidase C1

Eukaryotic thiol (cysteine) proteases active sites

cross-reference(s) THIOL_PROTEASE_CYS; THIOL_PROTEASE_HIS;

THIOL_PROTEASE_ASN

Eukaryotic thiol proteases (EC 3.4.22.-) [1] are a family of proteolytic enzymes which contain an active site cysteine. Catalysis proceeds through a thioester intermediate and is facilitated by a nearby histidine side chain; an asparagine completes the essential catalytic triad. The proteases which are currently known to belong to this family are listed below (references are only provided for recently determined sequences).

- Vertebrate lysosomal cathepsins B (EC 3.4.22.1), H (EC 3.4.22.16), L (EC 3.4.22.15), and S (EC 3.4.22.27) [2].

- Vertebrate lysosomal dipeptidyl peptidase I (EC 3.4.14.1) (also known as cathepsin C) [2].

- Vertebrate calpains (EC 3.4.22.17). Calpains are intracellular calcium-activated thiol protease that contain both a N-terminal catalytic domain and a C-terminal calcium-binding domain.

- Mammalian cathepsin K, which seems involved in osteoclastic bone resorption [3].

- Human cathepsin O [4].

- Bleomycin hydrolase. An enzyme that catalyzes the inactivation of the antitumor drug BLM (a glycopeptide).

- Plant enzymes: barley aleurain (EC 3.4.22.16), EP-B1/B4; kidney bean EP-C1, rice bean SH-EP; kiwi fruit actinidin (EC 3.4.22.14); papaya latex papain (EC 3.4.22.2), chymopapain (EC 3.4.22.6), caricain (EC 3.4.22.30), and proteinase IV (EC 3.4.22.25); pea turgor-responsive protein 15A; pineapple stem bromelain (EC 3.4.22.32); rape COT44; rice oryzain alpha, beta, and gamma; tomato low-temperature induced, Arabidopsis thaliana A494, RD19A and RD21A.

- House-dust mites allergens DerP1 and EurM1.

- Cathepsin B-like proteinases from the worms *Caenorhabditis elegans* (genes gcp-1, cpr-3, cpr-4, cpr-5 and cpr-6), *Schistosoma mansoni* (antigen SM31) and *Japonica* (antigen SJ31), *Haemonchus contortus* (genes AC-1 and AC-2), and *Ostertagia ostertagi* (CP-1 and CP-3).

- Slime mold cysteine proteinases CP1 and CP2.

- Cruzipain from *Trypanosoma cruzi* and *brucei*.

- Throphozoite cysteine proteinase (TCP) from various *Plasmodium* species.

- Proteases from *Leishmania mexicana*, *Theileria annulata* and *Theileria parva*.

- Baculoviruses cathepsin-like enzyme (v-cath).
 - Drosophila small optic lobes protein (gene sol), a neuronal protein that contains a calpain-like domain.
 - Yeast thiol protease BLH1/YCP1/LAP3.
- 5 - Caenorhabditis elegans hypothetical protein C06G4.2, a calpain-like protein.

Two bacterial peptidases are also part of this family:

- Aminopeptidase C from Lactococcus lactis (gene pepC) [5].
- 10 - Thiol protease tpr from Porphyromonas gingivalis.

Three other proteins are structurally related to this family, but may have lost their proteolytic activity.

- 15 - Soybean oil body protein P34. This protein has its active site cysteine replaced by a glycine.
 - Rat testin, a sertoli cell secretory protein highly similar to cathepsin L but with the active site cysteine is replaced by a serine. Rat testin should not be confused with mouse testin which is a LIM-domain protein (see <PDOC00382>).
 - 20 - Plasmodium falciparum serine-repeat protein (SERA), the major blood stage antigen. This protein of 111 Kd possesses a C-terminal thiol-protease-like domain [6], but the active site cysteine is replaced by a serine.
- The sequences around the three active site residues are well conserved and can be used as signature patterns.

25 Consensus pattern Q-x(3)-[GE]-x-C-[YW]-x(2)-[STAGC]-[STAGCV] [C is the active site residue]

Note the residue in position 4 of the pattern is almost always cysteine; the only exceptions are calpains (Leu), bleomycin hydrolase (Ser) and yeast YCP1 (Ser). Note the residue in position

30 5 of the pattern is always Gly except in papaya protease IV where it is Glu.

Consensus pattern [LIVMGSTAN]-x-H-[GSACE]-[LIVM]-x-[LIVMAT](2)-G-x-[GSADNH] [H is the active site residue]

Consensus pattern [FYCH]-[WI]-[LIVT]-x-[KROAG]-N-[ST]-W-x(3)-[FYW]-G-x(2)-G-[LFYW]-[LIVMFYG]-x-[LIVMF] [N is the active site residue]

Note these proteins belong to family C1 (papain-type) and C2 (calpains) in the classification of peptidases [7,E1].

[1]Dufour E. Biochimie 70:1335-1342(1988).

5 [2]Kirschke H., Barrett A.J., Rawlings N.D. Protein Prof. 2:1587-1643(1995).

[3]Shi G.-P., Chapman H.A., Bhairi S.M., Deleeuw C., Reddy V.Y., Weiss S.J. FEBS Lett. 357:129-134(1995).

[4]Velasco G., Ferrando A.A., Puente X.S., Sanchez L.M., Lopez-Otin C. J. Biol. Chem. 269:27136-27142(1994).

10 [5]Chapot-Chartier M.P., Nardi M., Chopin M.C., Chopin A., Gripon J.C. Appl. Environ. Microbiol. 59:330-333(1993).

[6]Higgins D.G., McConnell D.J., Sharp P.M. Nature 340:604-604(1989).

[7]Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:461-486(1994).

15

746. Peptidase M22

Glycoprotease family signature cross-reference(s) GLYCOPROTEASE

Glycoprotease (GCP) (EC 3.4.24.57) [1], or o-sialoglycoprotein endopeptidase,

is a metalloprotease secreted by *Pasteurella haemolytica* which specifically

20 cleaves G-sialoglycoproteins such as glycophorin A. The sequence of GCP is highly similar to the following uncharacterized proteins:

- *Escherichia coli* hypothetical protein ygiD (ORF-X).

- *Bacillus subtilis* hypothetical protein ydiE.

25 - *Mycobacterium leprae* hypothetical protein U229E.

- *Mycobacterium tuberculosis* hypothetical protein MtCY78.10.

- *Synechocystis* strain PCC 6803 hypothetical protein slr0807.

- *Methanococcus jannaschii* hypothetical protein MJ1130.

- *Haloarcula marismortui* hypothetical protein in HSH 3'region.

30 - Yeast hypothetical protein YKR038c.

- Yeast hypothetical protein QRI7.

One of the conserved regions contains two conserved histidines. It is possible that this region is involved in coordinating a metal ion such as zinc.

Consensus pattern[KR]-[GSAT]-x(4)-[FYWLH]-[DQNGK]-x-P-x-[LIVMFY]-x(3)-H-x(2)-[AG]-H-[LIVM]

Note these proteins belong to family M22 in the classification of

5 peptidases [2,E1].

[1]Abdullah K.M., Lo R.Y.C., Mellors A. J. Bacteriol. 173:5597-5603(1991).

[2]Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

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747. SAM. SAM domain (Sterile alpha motif)

It has been suggested that SAM is an evolutionarily conserved protein binding domain that is involved in the regulation of numerous developmental processes in diverse eukaryotes. The SAM domain can potentially function as a protein interaction module through its ability to

15 homo- and heterooligomerise with other SAM domains. Number of members: 81

[1]Medline: 96100659 SAM: A novel motif in yeast sterile alpha and Drosophila polyhomeotic proteins Ponting CP; Prot Sci 1995;4:1928-1930.

20

[2]Medline: 97160498 SAM as a protein interaction domain involved in developmental regulation. Shultz J, Ponting CP, Hofmann K, Bork P; Prot Sci 1997;6:249-253.

[3]Medline: 99101382 The crystal structure of an Eph receptor SAM domain reveals a mechanism for modular dimerization. Reference Author: Stapleton D, Balan I, Pawson T, Sicheri F; Nat Struct Biol 1999;6:44-49.

25

748. Tyrosinase signatures cross-reference(s) TYROSINASE_1; TYROSINASE_2

Tyrosinase (EC 1.14.18.1) [1] is a copper monooxygenases that catalyzes the hydroxylation of monophenols and the oxidation of o-diphenols to o-quinols.

This enzyme, found in prokaryotes as well as in eukaryotes, is involved in the

30 formation of pigments such as melanins and other polyphenolic compounds.

Tyrosinase binds two copper ions (CuA and CuB). Each of the two copper ion has been shown [2] to be bound by three conserved histidines residues. The regions around these copper-binding ligands are well conserved and also shared by some

hemocyanins, which are copper-containing oxygen carriers from the hemolymph of many molluscs and arthropods [3,4].

At least two proteins related to tyrosinase are known to exist in mammals:

- TRP-1 (TYRP1) [5], which is responsible for the conversion of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) to indole-5,6-quinone-2-carboxylic acid.
- TRP-2 (TYRP2) [6], which is the melanogenic enzyme DOPachrome tautomerase (EC 5.3.3.12) that catalyzes the conversion of DOPachrome to DHICA. TRP-2 differs from tyrosinases and TRP-1 in that it binds two zinc ions instead of copper [7].

Other proteins that belong to this family are:

- Plants polyphenol oxidases (PPO) (EC 1.10.3.1) which catalyze the oxidation of mono- and o-diphenols to o-diquinones [8].
- *Caenorhabditis elegans* hypothetical protein C02C2.1.

Two signature patterns for tyrosinase and related proteins have been derived

The first one contains two of the histidines that bind CuA, and is located in the N-terminal section of tyrosinase. The second pattern contains a histidine that binds CuB, that pattern is located in the central section of the enzyme.

Consensus pattern H-x(4,5)-F-[LIVMFTP]-x-[FW]-H-R-x(2)-[LM]-x(3)-E

[The two H's are copper ligands]

Consensus pattern D-P-x-F-[LIVMFYW]-x(2)-H-x(3)-D [H is a copper ligand]

[1] Lerch K. Prog. Clin. Biol. Res. 256:85-98(1988).

[2] Jackman M.P., Hajnal A., Lerch K. Biochem. J. 274:707-713(1991).

[3] Linzen B. Naturwissenschaften 76:206-211(1989).

[4] Lang W.H., van Holde K.E. Proc. Natl. Acad. Sci. U.S.A. 88:244-248(1991).

[5] Kobayashi T., Urabe K., Winder A., Jimenez-Cervantes C., Imokawa G., Brewington T., Solano F., Garcia-Borron J.C., Hearing V.J. EMBO J. 13:5818-5825(1994).

[6]Jackson I.J., Chambers D.M., Tsukamoto K., Copeland N.G., Gilbert D.J., Jenkins N.A., Hearing V. EMBO J. 11:527-535(1992).

[7]Solano F., Martinez-Liarte J.H., Jimenez-Cervantes C., Garcia-Borrón J.C., Lozano J.A. Biochem. Biophys. Res. Commun. 204:1243-1250(1994).

5 [8]Cary J.W., Lax A.R., Flurkey W.H. Plant Mol. Biol. 20:245-253(1992).

749. (Mur Ligase) Folylpolyglutamate synthase signatures

10 Folylpolyglutamate synthase (EC 6.3.2.17) (FPGS) [1] is the enzyme of folate metabolism that catalyzes ATP-dependent addition of glutamate moieties to tetrahydrofolate.

Its sequence is moderately conserved between prokaryotes (gene folC) and eukaryotes. We developed two signature patterns based on the conserved regions which are rich in glycine residues and could play a role in the catalytical
15 activity and/or in substrate binding.

Description of pattern(s) and/or profile(s)

Consensus pattern[LIVMFY]-x-[LIVM]-[STAG]-G-T-[NK]-G-K-x-[ST]-x(7)-[LIVM](2)-x(3)-[GSK]

20 Consensus pattern[LIVMFY](2)-E-x-G-[LIVM]-[GA]-G-x(2)-D-x-[GST]-x-[LIVM](2)

[1]Shane B., Garrow T., Brenner A., Chen L., Choi Y.J., Hsu J.C., Stover P. Adv. Exp. Med. Biol. 338:629-634(1993).

750. (Peptidase M3) Neutral zinc metallopeptidases, zinc-binding region signature

The majority of zinc-dependent metallopeptidases (with the notable exception of the carboxypeptidases) share a common pattern of primary structure [1,2,3] in the part of their sequence involved in the binding of zinc, and can be grouped together as a
30 superfamily, known as the metzincins, on the basis of this sequence similarity. They can be classified into a number of distinct families [4, E1] which are listed below along with the proteases which are currently known to belong to these families.

Family M1

- Bacterial aminopeptidase N (EC 3.4.11.2) (gene pepN).
- Mammalian aminopeptidase N (EC 3.4.11.2).
- Mammalian glutamyl aminopeptidase (EC 3.4.11.7) (aminopeptidase A). It may play a role in regulating growth and differentiation of early B-lineage cells.
- 5 - Yeast aminopeptidase yscII (gene APE2).
- Yeast alanine/arginine aminopeptidase (gene AAP1).
- Yeast hypothetical protein YIL137c.
- Leukotriene A-4 hydrolase (EC 3.3.2.6). This enzyme is responsible for the hydrolysis of an epoxide moiety of LTA-4 to form LTB-4; it has been shown that it binds zinc and is
- 10 capable of peptidase activity.

Family M2

- Angiotensin-converting enzyme (EC 3.4.15.1) (dipeptidyl carboxypeptidase I) (ACE) the enzyme responsible for hydrolyzing angiotensin I to angiotensin II. There are two forms
- 15 of ACE: a testis-specific isozyme and a somatic isozyme which has two active centers.

Family M3

- Thimet oligopeptidase (EC 3.4.24.15), a mammalian enzyme involved in the cytoplasmic degradation of small peptides.
- 20 - Neurolysin (EC 3.4.24.16) (also known as mitochondrial oligopeptidase M or microsomal endopeptidase).
- Mitochondrial intermediate peptidase precursor (EC 3.4.24.59) (MIP). It is involved the second stage of processing of some proteins imported in the mitochondrion.
- Yeast saccharolysin (EC 3.4.24.37) (proteinase yscD).
- 25 - Escherichia coli and related bacteria dipeptidyl carboxypeptidase (EC 3.4.15.5) (gene dcp).
- Escherichia coli and related bacteria oligopeptidase A (EC 3.4.24.70) (gene opdA or prlC).
- Yeast hypothetical protein YKL134c.

Family M4

- 30 - Thermostable thermolysins (EC 3.4.24.27), and related thermolabile neutral proteases (bacillolysins) (EC 3.4.24.28) from various species of Bacillus.
- Pseudolysin (EC 3.4.24.26) from Pseudomonas aeruginosa (gene lasB).
- Extracellular elastase from Staphylococcus epidermidis.

- Extracellular protease prt1 from *Erwinia carotovora*.
- Extracellular minor protease smp from *Serratia marcescens*.
- Vibriolysin (EC 3.4.24.25) from various species of *Vibrio*.
- Protease prtA from *Listeria monocytogenes*.
- 5 - Extracellular proteinase proA from *Legionella pneumophila*.

Family M5

- Mycolysin (EC 3.4.24.31) from *Streptomyces cacaoi*.

10 Family M6

- Immune inhibitor A from *Bacillus thuringiensis* (gene ina). Ina degrades two classes of insect antibacterial proteins, attacins and cecropins.

Family M7

- 15 - *Streptomyces* extracellular small neutral proteases

Family M8

- Leishmanolysin (EC 3.4.24.36) (surface glycoprotein gp63), a cell surface protease from various species of *Leishmania*.

20 Family M9

- Microbial collagenase (EC 3.4.24.3) from *Clostridium perfringens* and *Vibrio alginolyticus*.

25 Family M10A

- Serralyisin (EC 3.4.24.40), an extracellular metalloprotease from *Serratia*.
- Alkaline metalloproteinase from *Pseudomonas aeruginosa* (gene aprA).
- Secreted proteases A, B, C and G from *Erwinia chrysanthemi*.
- Yeast hypothetical protein YIL108w.

30 Family M10B

- Mammalian extracellular matrix metalloproteinases (known as matrixins) [5]: MMP-1 (EC 3.4.24.7) (interstitial collagenase), MMP-2 (EC 3.4.24.24) (72 Kd gelatinase), MMP-9 (EC 3.4.24.35) (92 Kd gelatinase), MMP-7 (EC 3.4.24.23) (matrylisin), MMP-8 (EC 3.4.24.34)

(neutrophil collagenase), MMP-3 (EC 3.4.24.17) (stromelysin-1), MMP-10 (EC 3.4.24.22) (stromelysin-2), and MMP-11 (stromelysin-3), MMP-12 (EC 3.4.24.65) (macrophage metalloelastase).

- Sea urchin hatching enzyme (envelysin) (EC 3.4.24.12). A protease that allows the embryo to digest the protective envelope derived from the egg extracellular matrix.
- Soybean metalloendoproteinase 1.

Family M11

- *Chlamydomonas reinhardtii* gamete lytic enzyme (GLE).

Family M12A

- Astacin (EC 3.4.24.21), a crayfish endoprotease.
- Mepirin A (EC 3.4.24.18), a mammalian kidney and intestinal brush border metalloendopeptidase.
- Bone morphogenic protein 1 (BMP-1), a protein which induces cartilage and bone formation and which expresses metalloendopeptidase activity. The *Drosophila* homolog of BMP-1 is the dorsal-ventral patterning protein tolloid.
- Blastula protease 10 (BP10) from *Paracentrotus lividus* and the related protein SpAN from *Strongylocentrotus purpuratus*.
- *Caenorhabditis elegans* protein toh-2.
- *Caenorhabditis elegans* hypothetical protein F42A10.8.
- Choriolytins L and H (EC 3.4.24.67) (also known as embryonic hatching proteins LCE and HCE) from the fish *Oryzias latipes*. These proteases participate in the breakdown of the egg envelope, which is derived from the egg extracellular matrix, at the time of hatching.

Family M12B

- Snake venom metalloproteinases [6]. This subfamily mostly groups proteases that act in hemorrhage. Examples are: adamalysin II (EC 3.4.24.46), atrolysin C/D (EC 3.4.24.42), atrolysin E (EC 3.4.24.44), fibrolase (EC 3.4.24.72), trimerelysin I (EC 3.4.25.52) and II (EC 3.4.25.53).
- Mouse cell surface antigen MS2.

Family M13

- Mammalian neprilysin (EC 3.4.24.11) (neutral endopeptidase) (NEP).
- Endothelin-converting enzyme 1 (EC 3.4.24.71) (ECE-1), which process the precursor of endothelin to release the active peptide.
- Kell blood group glycoprotein, a major antigenic protein of erythrocytes. The Kell protein is very probably a zinc endopeptidase.
- Peptidase O from *Lactococcus lactis* (gene pepO).

Family M27

- Clostridial neurotoxins, including tetanus toxin (TeTx) and the various botulinum toxins (BoNT). These toxins are zinc proteases that block neurotransmitter release by proteolytic cleavage of synaptic proteins such as synaptobrevins, syntaxin and SNAP-25 [7,8].

Family M30

- *Staphylococcus hyicus* neutral metalloprotease.

Family M32

- Thermostable carboxypeptidase 1 (EC 3.4.17.19) (carboxypeptidase Taq), an enzyme from *Thermus aquaticus* which is most active at high temperature.

Family M34

- Lethal factor (LF) from *Bacillus anthracis*, one of the three proteins composing the anthrax toxin.

Family M35

- Deuterolysin (EC 3.4.24.39) from *Penicillium citrinum* and related proteases from various species of *Aspergillus*.

Family M36

- Extracellular elastinolytic metalloproteinases from *Aspergillus*.

From the tertiary structure of thermolysin, the position of the residues acting as zinc ligands and those involved in the catalytic activity are known. Two of the zinc ligands are histidines which are very close together in the sequence; C-terminal to the first histidine is

a glutamic acid residue which acts as a nucleophile and promotes the attack of a water molecule on the carbonyl carbon of the substrate. A signature pattern which includes the two histidine and the glutamic acid residues is sufficient to detect this superfamily of proteins.

5

Description of pattern(s) and/or profile(s)

Consensus pattern[GSTALIVN]-x(2)-H-E-[LIVMFYW]-{DEHRKP}-H-x-

[LIVMFYWGSPO] [The

two H's are zinc ligands] [E is the active site residue]

- 10 Sequences known to belong to this class detected by the patternALL,
except for members of families M5, M7 and M11.

Other sequence(s) detected in SWISS-PROT55; including Neurospora
crassa conidiation-specific protein 13 which could be a
zinc-protease.

- 15 [1]Jongeneel C.V., Bouvier J., Bairoch A.

FEBS Lett. 242:211-214(1989).

[2]Murphy G.J.P., Murphy G., Reynolds J.J.

FEBS Lett. 289:4-7(1991).

[3]Bode W., Grams F., Reinemer P., Gomis-Rueth F.-X., Baumann U., McKay

- 20 D.B., Stoecker W.

Zoology 99:237-246(1996).

[4]Rawlings N.D., Barrett A.J.

Meth. Enzymol. 248:183-228(1995).

[5]Woessner J. Jr.

- 25 FASEB J. 5:2145-2154(1991).

[6]Hite L.A., Fox J.W., Bjarnason J.B.

[7]Montecucco C., Schiavo G.

Trends Biochem. Sci. 18:324-327(1993).

[8]Niemann H., Blasi J., Jahn R.

- 30 Trends Cell Biol. 4:179-185(1994).

- tRNA pseudouridine synthase is involved in the formation of pseudouridine at the anticodon stem and loop of transfer-RNAs. Pseudouridine is an isomer of uridine (5-(beta-D-ribofuranosyl) uracil), and is the most abundant modified nucleoside found in all cellular RNAs. The TruA-like proteins also exhibit a conserved sequence with a strictly conserved aspartic acid, likely involved in catalysis. Number of members: 25

[1] Medline: 98254513. Transfer RNA-pseudouridine synthetase PusI of *Saccharomyces cerevisiae* contains one atom of zinc essential for its native conformation and tRNA recognition. Arluison V, Hountondji C, Robert B, Grosjean H; *Biochemistry* 1998;37:7268-7276.

752. EPSP synthase signatures

- EPSP synthase (3-phosphoshikimate 1-carboxyvinyltransferase) (EC 2.5.1.19) catalyzes the sixth step in the biosynthesis from chorismate of the aromatic amino acids (the shikimate pathway) in bacteria (gene *aroA*), plants and fungi (where it is part of a multifunctional enzyme which catalyzes five consecutive steps in this pathway) [1]. EPSP synthase has been extensively studied as it is the target of the potent herbicide glyphosate which inhibits the enzyme.

- The sequence of EPSP from various biological sources shows that the structure of the enzyme has been well conserved throughout evolution. Two conserved regions were selected as signature patterns. The first pattern corresponds to a region that is part of the active site and which is also important for the resistance to glyphosate [2]. The second pattern is located in the C-terminal part of the protein and contains a conserved lysine which seems to be important for the activity of the enzyme.

Description of pattern(s) and/or profile(s)

- 30 Consensus pattern[LIVM]-x(2)-[GN]-N-[SA]-G-T-[STA]-x-R-x-[LIVMY]-x-[GSTA]
Consensus pattern[KR]-x-[KH]-E-[CST]-[DNE]-R-[LIVM]-x-[STA]-[LIVMC]-x(2)-[EN]-
[LIVMF]-x-[KRA]-[LIVMF]-G

[1]Stallings W.C., Abdel-Megid S.S., Lim L.W., Shieh H.-S., Dayringer H.E., Leimgruber N.K., Stegeman R.A., Anderson K.S., Sikorski J.A., Padgett S.R., Kishore G.M. Proc. Natl. Acad. Sci. U.S.A. 88:5046-5050(1991).

- 5 [2]Padgett S.R., Re D.B., Gaser C.S., Eicholtz D.A., Frazier R.B., Hironaka C.M., Levine E.B., Shah D.M., Fraley R.T., Kishore G.M. J. Biol. Chem. 266:22364-22369(1991).

753. Glyco_hydro_18

Glycosyl hydrolases family 18. Number of members: 173

- 10 [1]Medline: 95219379. Crystal structure of a bacterial chitinase at 2.3 Å resolution. Perrakis A, Tews I, Dauter Z, Oppenheim AB, Chet I, Wilson KS, Vorgias CE; Structure 1994;2:1169-1180.

15 754. Esterase

Putative esterase

This family contains Esterase D Swiss:P10768. However it is not clear if all members of the family have the same function. This family is possibly related to the COesterase family.

Number of members: 36

20

755. (HMA) Heavy-metal-associated domain

A conserved domain of about 30 amino acid residues has been found [1] in a number of proteins that transport or detoxify heavy metals. This domain contains two conserved cysteines that could be involved in the binding of these metals. The domain has been termed Heavy-Metal-Associated (HMA). It has been found in:

- A variety of cation transport ATPases (E1-E2 ATPases) (see <PDOC00139>). The human copper ATPases ATP7A and ATP7B which are respectively involved in Menke's and Wilson's diseases. ATP7A and ATP7B both contain 6 tandem copies of the HMA domain. The copper ATPases CCC2 from budding yeast, copA from Enterococcus faecalis and synA from Synechococcus contain one copy of the HMA domain. The cadmium ATPases cadA from Bacillus firmus and from plasmid pI258 from Staphylococcus aureus also contain a single HMA domain, while a chromosomal Staphylococcus aureus cadA contains two copies. Other, less characterized ATPases
- 30

that contain the HMA domain are: fixI from *Rhizobium meliloti*, pacS from *Synechococcus* strain PCC 7942), *Mycobacterium leprae* ctpA and ctpB and *Escherichia coli* hypothetical protein yhhO. In all these ATPases the HMA domain(s) are located in the N-terminal section.

- 5 - Mercuric reductase (EC 1.16.1.1) (gene merA) which is generally encoded by plasmids carried by mercury-resistant Gram-negative bacteria. Mercuric reductase is a class-1 pyridine nucleotide-disulphide oxidoreductase (see <PDOC00073>). There is generally one HMA domain (with the exception of a chromosomal merA from *Bacillus* strain RC607 which has two) in the N-terminal part of merA.
- 10 - Mercuric transport protein periplasmic component (gene merP), also encoded by plasmids carried by mercury-resistant Gram-negative bacteria. It seems to be a mercury scavenger that specifically binds to one Hg(2+) ion and which passes it to the mercuric reductase via the merT protein. The N-terminal half of merP is a HMA domain.
- 15 - *Helicobacter pylori* copper-binding protein copP.
- Yeast protein ATX1 [2], which could act in the transport and/or partitioning of copper.

The consensus pattern for HMA spans the complete domain.

Description of pattern(s) and/or profile(s)

Consensus pattern[LIVN]-x(2)-[LIVMFA]-x-C-x-[STAGCDNH]-C-x(3)-[LIVFG]-x(3)-[LIV]-x(9,11)-[IVA]-x-[LVFYS] [The two C's probably bind metals]

[1] Bull P.C., Cox D.W. Trends Genet. 10:246-252(1994).

[2] Lin S.-J., Culotta V.L. Proc. Natl. Acad. Sci. U.S.A. 92:3784-3788(1995).

756. (Peptidase M10) Matrixins cysteine switch

PROSITE cross-reference(s): CYSTEINE_SWITCH

30 Mammalian extracellular matrix metalloproteinases (EC 3.4.24.-), also known as matrixins [1] (see <PDOC00129>), are zinc-dependent enzymes. They are secreted by cells in an inactive form (zymogen) that differs from the mature enzyme by the presence of an N-terminal propeptide. A highly conserved octapeptide is found two residues downstream of the C-terminal end of the propeptide. This region has been shown to be involved in

autoinhibition of matrixins [2,3]; a cysteine within the octapeptide chelates the active site zinc ion, thus inhibiting the enzyme. This region has been called the 'cysteine switch' or 'autoinhibitor region'.

A cysteine switch has been found in the following zinc proteases:

- 5
- MMP-1 (EC 3.4.24.7) (interstitial collagenase).
- MMP-2 (EC 3.4.24.24) (72 Kd gelatinase).
- MMP-3 (EC 3.4.24.17) (stromelysin-1).
- MMP-7 (EC 3.4.24.23) (matrilysin).
- 10 - MMP-8 (EC 3.4.24.34) (neutrophil collagenase).
- MMP-9 (EC 3.4.24.35) (92 Kd gelatinase).
- MMP-10 (EC 3.4.24.22) (stromelysin-2).
- MMP-11 (EC 3.4.24.-) (stromelysin-3).
- MMP-12 (EC 3.4.24.65) (macrophage metalloelastase).
- 15 - MMP-13 (EC 3.4.24.-) (collagenase 3).
- MMP-14 (EC 3.4.24.-) (membrane-type matrix metalloproteinase 1).
- MMP-15 (EC 3.4.24.-) (membrane-type matrix metalloproteinase 2).
- MMP-16 (EC 3.4.24.-) (membrane-type matrix metalloproteinase 3).
- Sea urchin hatching enzyme (EC 3.4.24.12) (envelysin) [4].
- 20 - Chlamydomonas reinhardtii gamete lytic enzyme (GLE) [5].

Description of pattern(s) and/or profile(s)

Consensus patternP-R-C-[GN]-x-P-[DR]-[LIVSAPKQ] [C chelates the zinc ion]

- 25 [1]Woessner J. Jr. FASEB J. 5:2145-2154(1991).
- [2]Sanchez-Lopez R., Nicholson R., Gesnel M.C., Matrisian L.M., Breathnach R. J. Biol. Chem. 263:11892-11899(1988).
- [3]Park A.J., Matrisian L.M., Kells A.F., Pearson R., Yuan Z., Navre M. J. Biol. Chem. 266:1584-1590(1991).
- 30 [4]Lepage T., Gache C. EMBO J. 9:3003-3012(1990).
- [5]Kinoshta T., Fukuzawa H., Shimada T., Saito T., Matsuda Y. Proc. Natl. Acad. Sci. U.S.A. 89:4693-4697(1992).

757. (Peptidase S8) Serine proteases, subtilase family, active sites

PROSITE cross-reference(s): PS00136; SUBTILASE_ASP, PS00137; SUBTILASE_HIS, PS00138; SUBTILASE_SER

Subtilases [1,2] are an extensive family of serine proteases whose catalytic activity is

- 5 provided by a charge relay system similar to that of the trypsin family of serine proteases but which evolved by independent convergent evolution. The sequence around the residues involved in the catalytic triad (aspartic acid, serine and histidine) are completely different from that of the analogous residues in the trypsin serine proteases and can be used as signatures specific to that category of proteases.

- 10 The subtilase family currently includes the following proteases:

- Subtilisins (EC 3.4.21.62), these alkaline proteases from various *Bacillus* species have been the target of numerous studies in the past thirty years.

- Alkaline elastase YaB from *Bacillus* sp. (gene ale).

- Alkaline serine exoprotease A from *Vibrio alginolyticus* (gene proA).

- 15 - Aqualysin I from *Thermus aquaticus* (gene pstI).

- AspA from *Aeromonas salmonicida*.

- Bacillopeptidase F (esterase) from *Bacillus subtilis* (gene bpf).

- CSA peptidase from *Streptococcus pyogenes* (gene scpA).

- Cell envelope-located proteases PI, PII, and PIII from *Lactococcus lactis*.

- 20 - Extracellular serine protease from *Serratia marcescens*.

- Extracellular protease from *Xanthomonas campestris*.

- Intracellular serine protease (ISP) from various *Bacillus*.

- Minor extracellular serine protease epr from *Bacillus subtilis* (gene epr).

- Minor extracellular serine protease vpr from *Bacillus subtilis* (gene vpr).

- 25 - Nisin leader peptide processing protease nisP from *Lactococcus lactis*.

- Serotype-specific antigene 1 from *Pasteurella haemolytica* (gene ssa1).

- Thermitase (EC 3.4.21.66) from *Thermoactinomyces vulgaris*.

- Calcium-dependent protease from *Anabaena variabilis* (gene prcA).

- Halolysin from halophilic bacteria sp. 172p1 (gene hly).

- 30 - Alkaline extracellular protease (AEP) from *Yarrowia lipolytica* (gene xpr2).

- Alkaline proteinase from *Cephalosporium acremonium* (gene alp).

- Cerevisin (EC 3.4.21.48) (vacuolar protease B) from yeast (gene PRB1).

- Cuticle-degrading protease (pr1) from *Metarhizium anisopliae*.

- KEX-1 protease from *Kluyveromyces lactis*.

- Kexin (EC 3.4.21.61) from yeast (gene KEX-2).
- Oryzin (EC 3.4.21.63) (alkaline proteinase) from *Aspergillus* (gene alp).
- Proteinase K (EC 3.4.21.64) from *Tritirachium album* (gene proK).
- Proteinase R from *Tritirachium album* (gene proR).

- 5 - Proteinase T from *Tritirachium album* (gene proT).
- Subtilisin-like protease III from yeast (gene YSP3).
 - Thermomycolin (EC 3.4.21.65) from *Malbranchea sulfurea*.
 - Furin (EC 3.4.21.85), neuroendocrine convertases 1 to 3 (NEC-1 to -3) and PACE4 protease from mammals, other vertebrates, and invertebrates. These proteases are involved
- 10 in the processing of hormone precursors at sites comprised of pairs of basic amino acid residues [3].

- Tripeptidyl-peptidase II (EC 3.4.14.10) (tripeptidyl aminopeptidase) from Human.
 - Prestalk-specific proteins tagB and tagC from slime mold [4]. Both proteins consist of two domains: a N-terminal subtilase catalytic domain and a C-terminal ABC transporter domain
- 15 (see <PDOC00185>).

Description of pattern(s) and/or profile(s)

Consensus pattern[STAIV]-x-[LIVMF]-[LIVM]-D-[DSTA]-G-[LIVMFC]-x(2,3)-[DNH] [D is the active site residue]

- 20 Consensus patternH-G-[STM]-x-[VIC]-[STAGC]-[GS]-x-[LIVMA]-[STAGCLV]-[SAGM] [H is the active site residue]

Consensus patternG-T-S-x-[SA]-x-P-x(2)-[STAVC]-[AG] [S is the active site residue]

Note if a protein includes at least two of the three active site signatures, the probability of it being a serine protease from the subtilase family is 100%

- 25 Note these proteins belong to family S8 in the classification of peptidases [5,E1].

[1]Siezen R.J., de Vos W.M., Leunissen J.A.M., Dijkstra B.W. Protein Eng. 4:719-737(1991).

- 30 [2]Siezen R.J. (In) Proceeding subtilisin symposium, Hamburg, (1992).

[3]Barr P.J. Cell 66:1-3(1991).

[4]Shaulsky G., Kuspa A., Loomis W.F.; Genes Dev. 9:1111-1122(1995).

[5]Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

758. (SSB) Single-strand binding protein family signatures

PROSITE cross-reference(s): PS00735; SSB_1, PS00736; SSB_2

The *Escherichia coli* single-strand binding protein [1] (gene *ssb*), also known as the helix-destabilizing protein, is a protein of 177 amino acids. It binds tightly, as a homotetramer, to single-stranded DNA (ss-DNA) and plays an important role in DNA replication, recombination and repair.

Closely related variants of SSB are encoded in the genome of a variety of large self-transmissible plasmids. SSB has also been characterized in bacteria such as *Proteus mirabilis* or *Serratia marcescens*.

Eukaryotic mitochondrial proteins that bind ss-DNA and are probably involved in mitochondrial DNA replication are structurally and evolutionarily related to prokaryotic SSB.

Proteins currently known to belong to this subfamily are listed below [2].

- Mammalian protein Mt-SSB (P16).
- *Xenopus* Mt-SSBs and Mt-SSBr.
- *Drosophila* MtSSB.
- Yeast protein RIM1.

Two signature patterns have been developed for these proteins. The first is a conserved region in the N-terminal section of the SSB's. The second is a centrally located region which, in *Escherichia coli* SSB, is known to be involved in the binding of DNA.

25 Description of pattern(s) and/or profile(s)

Consensus pattern[LIVMF]-[NST]-[KRT]-[LIVM]-x-[LIVMF](2)-G-[NHRK]-[LIVM]-[GST]-x-[DET]

Consensus patternT-x-W-[HY]-[RNS]-[LIVM]-x-[LIVMF]-[FY]-[NGKR]

[1]Meyer R.R., Laine P.S. Microbiol. Rev. 54:342-380(1990).

[2]Stroumbakis N.D., Li Z., Tolia P.P. Gene 143:171-177(1994).

759. KDPG and KHG aldolases active site signatures

PROSITE cross-reference(s): ALDOLASE_KDPG_KHG_1, PS00160;
ALDOLASE_KDPG_KHG_2

4-hydroxy-2-oxoglutarate aldolase (EC 4.1.3.16) (KHG-aldolase) catalyzes the
interconversion of 4-hydroxy-2-oxoglutarate into pyruvate and glyoxylate. Phospho-2-
dehydro-3-deoxygluconate aldolase (EC 4.1.2.14) (KDPG-aldolase) catalyzes the
interconversion of 6-phospho-2-dehydro-3-deoxy-D-gluconate into pyruvate and
glyceraldehyde 3-phosphate.

These two enzymes are structurally and functionally related [1]. They are both homotrimeric
proteins of approximately 220 amino-acid residues. They are class I aldolases whose catalytic
mechanism involves the formation of a Schiff-base intermediate between the substrate and
the epsilon-amino group of a lysine residue. In both enzymes, an arginine is required for
catalytic activity.

Two signature patterns were developed for these enzymes. The first one contains the active
site arginine and the second, the lysine involved in the Schiff-base formation.

Description of pattern(s) and/or profile(s)

Consensus patternG-[LIVM]-x(3)-E-[LIV]-T-[LF]-R [R is the active site residue]
Consensus patternG-x(3)-[LIVMF]-K-[LF]-F-P-[SA]-x(3)-G [K is involved in Schiff-base
formation]

[1] Vlahos C J., Dekker E.E. J. Biol. Chem. 263:11683-11691(1988).

760. AP endonucleases family 1 signatures. PROSITE cross-reference(s): PS00726;
AP_NUCLEASE_F1_1, PS00727; AP_NUCLEASE_F1_2, PS00728;
AP_NUCLEASE_F1_3

DNA damaging agents such as the antitumor drugs bleomycin and neocarzinostatin or those
that generate oxygen radicals produce a variety of lesions in DNA. Amongst these is base-
loss which forms apurinic/apyrimidinic (AP) sites or strand breaks with atypical 3'termini.
DNA repair at the AP sites is initiated by specific endonuclease cleavage of the

phosphodiester backbone. Such endonucleases are also generally capable of removing blocking groups from the 3' terminus of DNA strand breaks.

AP endonucleases can be classified into two families on the basis of sequence similarity.

5 Family 1 groups the enzymes listed below [1].

- *Escherichia coli* exonuclease III (EC 3.1.11.2) (gene *xthA*).
- *Streptococcus pneumoniae* and *Bacillus subtilis* exonuclease A (gene *exoA*).
- Mammalian AP endonuclease 1 (AP1) (EC 4.2.99.18).
- 10 - *Drosophila* recombination repair protein 1 (gene *Rrp1*).
- *Arabidopsis thaliana* apurinic endonuclease-redox protein (gene *arp*).

Except for *Rrp1* and *arp*, these enzymes are proteins of about 300 amino-acid residues.

15 *Rrp1* and *arp* both contain additional and unrelated sequences in their N-terminal section (about 400 residues for *Rrp1* and 270 for *arp*).

Three signature patterns were developed for this family of enzymes. The patterns are based on the most conserved regions. The first pattern contains a glutamate which has been shown [2], in the *Escherichia coli* enzyme to bind a divalent metal ion such as magnesium or manganese

Consensus pattern[APF]-D-[LIVMF](2)-x-[LIVM]-Q-E-x-K [E binds a divalent metal ion]

Consensus patternD-[ST]-[FY]-R-[KH]-x(7,8)-[FYW]-[ST]-[FYW](2)

Consensus patternN-x-G-x-R-[LIVM]-D-[LIVMFYH]-x-[LV]-x-S

25

[1] Barzilay G., Hickson I.S. BioEssays 17:713-719(1995).

[2] Mol C.D., Kuo C.-F., Thayer M.M., Cunningham R.P., Tainer J.A. Nature 374:381-386(1995).

30 761. (ER)Enhancer of rudimentary signature, PROSITE cross-reference(s): PS01290; ER

The *Drosophila* protein 'enhancer of rudimentary' (gene *e(r)*) is a small protein of 104 residues whose function is not yet clear. From an evolutionary point of view, it is highly

conserved [1] and has been found to exist in probably all multicellular eukaryotic organisms. It has been proposed that this protein plays a role in the cell cycle.

A conserved region in the central part of the protein was selected as as signature pattern.

Consensus pattern Y-D-I-[SA]-x-L-[FY]-x-F-[IV]-D-x(3)-D-[LIV]-S

[1] Gelsthorpe M., Pulumati M., McCallum C., Dang-Vu K., Tsubota S.I. Gene 186:189-195(1997).

762. (ETF alpha) Electron transfer flavoprotein alpha-subunit signature, PROSITE cross-reference(s): PS00696; ETF_ALPHA

The electron transfer flavoprotein (ETF) [1,2] serves as a specific electron acceptor for various mitochondrial dehydrogenases. ETF transfers electrons to the main respiratory chain via ETF-ubiquinone oxidoreductase. ETF is an heterodimer that consist of an alpha and a beta subunit and which bind one molecule of FAD per dimer. A similar system also exists in some bacteria.

The alpha subunit of ETF is a protein of about 32 Kd which is structurally related to the bacterial nitrogen fixation protein fixB which could play a role in a redox process and feed electrons to ferredoxin.

Other related proteins are:

- Escherichia coli hypothetical protein ydiR.
- Escherichia coli hypothetical protein ygcQ.

A highly conserved region which is located in the C-terminal section was selected as a signature pattern for these proteins.

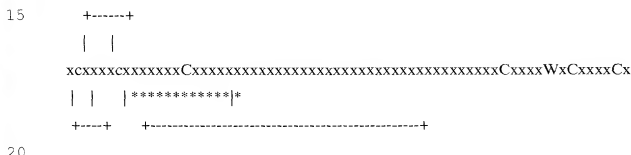
Consensus pattern [LI]-Y-[LIVM]-[AT]-x-G-[IV]-[SD]-G-x-[IV]-Q-H-x(2)-G-x(6)-[IV]-x-A-[IV]-N

- [2] Tsai M.H., Saier M.H. Jr. Res. Microbiol. 146:397-404(1995).

763. (lectin c) C-type lectin domain signature and profile

- 5 PROSITE cross-reference(s): PS00615; C_TYPE_LLECTIN_1, PS50041;
C TYPE LLECTIN 2

A number of different families of proteins share a conserved domain which was first characterized in some animal lectins and which seem to function as a calcium-dependent carbohydrate-recognition domain [1,2,3]. This domain, which is known as the C-type lectin domain (CTL) or as the carbohydrate-recognition domain (CRD), consists of about 110 to 130 residues. There are four cysteines which are perfectly conserved and involved in two disulfide bonds. A schematic representation of the CTL domain is shown below.



'C': conserved cysteine involved in a disulfide bond.

'c': optional cysteine involved in a disulfide bond.

'*': position of the pattern.

- 25 The categories of proteins, in which the CTL domain has been found, are listed below.

Type-II membrane proteins where the CTL domain is located at the C-terminal extremity of the proteins:

- 30 - Asialoglycoprotein receptors (ASGPR) (also known as hepatic lectins) [4]. The ASGPRs mediate the endocytosis of plasma glycoproteins to which the terminal sialic acid residue in their carbohydrate moieties has been removed.
- Low affinity immunoglobulin epsilon Fc receptor (lymphocyte IgE receptor), which plays an essential role in the regulation of IgE production and in the differentiation of B cells.

- Kupffer cell receptor. A receptor with an affinity for galactose and fucose, that could be involved in endocytosis.

- A number of proteins expressed on the surface of natural killer T-cells: NKG2, NKR-P1, YE1/88 (Ly-49), CD69 and on B-cells: CD72, LyB-2. The CTL- domain in these proteins is
- 5 distantly related to other CTL-domains; it is unclear whether they are likely to bind carbohydrates.

Proteins that consist of an N-terminal collagenous domain followed by a CTL- domain [5], these proteins are sometimes called 'collectins':

10

- Pulmonary surfactant-associated protein A (SP-A). SP-A is a calcium-dependent protein that binds to surfactant phospholipids and contributes to lower the surface tension at the air-liquid interface in the alveoli of the mammalian lung.

15

- Pulmonary surfactant-associated protein D (SP-D).

- Conglutinin, a calcium-dependent lectin-like protein which binds to a yeast cell wall extract and to immune complexes through the complement component (iC3b).

- Mannan-binding proteins (MBP) (also known as mannan-binding proteins).

20

MBP's bind mannanose and N-acetyl-D-glucosamine in a calcium-dependent manner.

- Bovine collectin-43 (CL-43).

25

Selectins (or LEC-CAM) [6,7]. Selectins are cell adhesion molecules implicated in the interaction of leukocytes with platelets or vascular endothelium. Structurally, selectins consist of a long extracellular domain, followed by a transmembrane region and a short cytoplasmic domain. The extracellular domain is itself composed of a CTL-domain, followed by an EGF-like domain and a variable number of SCR/Sushi repeats. Known selectins are:

30

- Lymph node homing receptor (also known as L-selectin, leukocyte adhesion molecule-1, (LAM-1), leu-8, gp90-mel, or LECAM-1)

- Endothelial leukocyte adhesion molecule 1 (ELAM-1, E-selectin or LECAM-2).

The ligand recognized by ELAM-1 is sialyl-Lewis x.

- Granule membrane protein 140 (GMP-140, P-selectin, PADGEM, CD62, or LECAM-3). The ligand recognized by GMP-140 is Lewis x.

Large proteoglycans that contain a CTL-domain followed by one copy of a SCR/ Sushi repeat, in their C-terminal section:

- Aggrecan (cartilage-specific proteoglycan core protein). This proteoglycan is a major component of the extracellular matrix of cartilagenous tissues where it has a role in the resistance to compression.

- Brevican.

- Neurocan.

- Versican (large fibroblast proteoglycan), a large chondroitin sulfate proteoglycan that may play a role in intercellular signalling.

In addition to the CTL and Sushi domains, these proteins also contain, in their N-terminal domain, an Ig-like V-type region, two or four link domains (see <PDOC00955>) and up to two EGF-like repeats.

Two type-I membrane proteins:

- Mannose receptor from macrophages. This protein mediates the endocytosis of glycoproteins by macrophages in several recognition and uptake processes. Its extracellular section consists of a fibronectin type II domain followed by eight tandem repeats of the CTL domain.

- 180 Kd secretory phospholipase A2 receptor (PLA2-R). A protein whose structure is highly similar to that of the mannose receptor.

- DEC-205 receptor. This protein is used by dendritic cells and thymic epithelial cells to capture and endocytose diverse carbohydrate-binding antigens and direct them to antigen-processing cellular compartments. DEC-

205 extracellular section consists of a fibronectin type II domain followed by ten tandem repeats of the CTL domain.

- Silk moth hemocytin, an humoral lectin which is involved in a self-defence mechanism. It is composed of 2 FA58C domains (see <PDOC00988>), a CTL domain, 2 VWFC domains (see <PDOC00928>), and a CTCK (see <PDOC00912>).

Various other proteins that uniquely consist of a CTL domain:

- 5 - Invertebrate soluble galactose-binding lectins. A category to which belong a humoral lectin from a flesh fly; echinoidin, a lectin from the coelomic fluid of a sea urchin; BRA-2 and BRA-3, two lectins from the coelomic fluid of a barnacle, a lectin from the tunicate *Polyandrocarpa misakiensis* and a newt oviduct lectin. The physiological importance of these lectins is not yet known but they may play an important role in defense mechanisms.
- 10 - Pancreatic stone protein (PSP) (also known as pancreatic thread protein (PTP), or reg), a protein that might act as an inhibitor of spontaneous calcium carbonate precipitation.
- Pancreatitis associated protein (PAP), a protein that might be involved in the control of bacterial proliferation.
- 15 - Tetranectin, a plasma protein that binds to plasminogen and to isolated kringles 4.
- Eosinophil granule major basic protein (MBP), a cytotoxic protein.
- A galactose specific lectin from a rattlesnake.
- Two subunits of a coagulation factor IX/factor X-binding protein (IX/X-bp),
- 20 a snake venom anticoagulant protein which binds with factors IX and X in the presence of calcium.
- Two subunits of a phospholipase A2 inhibitor from the plasma of a snake (PLI-A and PLI-B).
- A lipopolysaccharide-binding protein (LPS-BP) from the hemolymph of a
- 25 cockroach [8].
- Sea raven antifreeze protein (AFP) [9].

As a signature pattern for this domain, the C-terminal region with its three conserved cysteines was selected.

30 Consensus pattern C-[LIVMFYATG]-x(5,12)-[WL]-x-[DNSR]-x(2)-C-x(5,6)-[FYWLIVSTA]-[LIVMSTA]-C [The three C's are involved in disulfide bonds]

Note all CTL domains have five Trp residues before the second Cys,

with the exception of tunicate lectin and cockroach LPS-BP which have Leu.

Note this documentation entry is linked to both a signature pattern and a profile. As the profile is much more sensitive than the pattern, you should use it if you have access to the necessary software tools to do so.

- [1] Drickamer K. J. Biol. Chem. 263:9557-9560(1988).
- 10 [2] Drickamer K. Prog. Nucleic Acid Res. Mol. Biol. 45:207-232(1993).
- [3] Drickamer K. Curr. Opin. Struct. Biol. 3:393-400(1993).
- [4] Spiess M. Biochemistry 29:10009-10018(1990).
- [5] Weis W.I., Kahn R., Fourme R., Drickamer K., Hendrickson W.A. Science 254:1608-1615(1991).
- 15 [6] Siegelman M. Curr. Biol. 1:125-128(1991).
- [7] Lasky L.A. Science 238:964-969(1992).
- [8] Jomori T., Natori S. J. Biol. Chem. 266:13318-13323(1991).
- [9] Ng N.F.L., Hew C.-L. J. Biol. Chem. 267:16069-16075(1992).
- 20 764. (SRCR) Speract receptor repeated domain signature
PROSITE cross-reference(s): PS00420; SPERACT_RECEPTOR,

- The receptor for the sea urchin egg peptide speract is a transmembrane glycoprotein of 500 amino acid residues [1]. Structurally it consists of a large extracellular domain of 450 residues, followed by a transmembrane region and a small cytoplasmic domain of 12 amino acids. The extracellular domain contains four repeats of a 115 amino acids domain. There are 17 positions that are perfectly conserved in the four repeats, among them are six cysteines, six glycines, and three glutamates.
- 30 Such a domain is also found, once, in the C-terminal section of mammalian macrophage scavenger receptor type I [2], a membrane glycoproteins implicated in the pathologic deposition of cholesterol in arterial walls during atherogenesis.

The signature pattern that was derived spans part of the N-terminal section of the domain and contains 8 of the 17 conserved residues.

Consensus pattern G-x(5)-G-x(2)-E-x(6)-W-G-x(2)-C-x(3)-[FYW]-x(8)-C-x(3)-G

5

[1] Dangott J.J., Jordan J.E., Bellet R.A., Garbers D.L. Proc. Natl. Acad. Sci. U.S.A. 86:2128-2132(1989).

[2] Freeman M., Ashkenas J., Rees D.J., Kingsley D.M., Copeland N.G., Jenkins N.A., Krieger M. Proc. Natl. Acad. Sci. U.S.A. 87:8810-8814(1990).

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765. Bac_surface_Ag

Bacterial surface antigen

This entry includes the following surface antigens; D15 antigen from H.influenzae, OMA87 from P.multocida, OMP85 from N.meningitidis and N.gonorrhoeae. Number of members:

15

14

[1] Medline: 95255676. The sequencing of the 80-kDa D15 protective surface antigen of Haemophilus influenzae. Flack FS, Loosmore S, Chong P, Thomas WR; Gene 1995;156:97-99.

20

[2] Medline: 96333354. Cloning, sequencing, expression, and protective capacity of the oma87 gene encoding the Pasteurella multocida 87-kilodalton outer membrane antigen. Ruffolo CG, Adler B; Infect Immun 1996;64:3161-3167.

766. BRCA1 C Terminus (BRCT) domain

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The BRCT domain is found predominantly in proteins involved in cell cycle checkpoint functions responsive to DNA damage. It has been suggested that the Retinoblastoma protein contains a divergent BRCT domain, this has not been included in this family. The BRCT domain of XRCC1 forms a homodimer in the crystal structure Medline:99016060. This suggests that pairs of BRCT domains

30

associate as homo- or heterodimers. Number of members: 131

[1] Medline: 96259550. BRCA1 protein products ...Functional motifs... Koonin EV, Altschul SF, Bork P; Nature Genet 1996;13:266-268.

[2] Medline: 97153217. From BRCA1 to RAP1: A widespread BRCT module closely associated with DNA repair Callebaut I, Mornon JP; Febs lett 1997;400:25-30.

[3] Medline: 97186552. A superfamily of conserved domains in DNA damage responsive cell cycle checkpoint proteins Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin

5 EV; Faseb J 1997;11:68-76.

[4] Medline: 97402527. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ; Nucleic Acids Res 1997;25:3389-3402.

10 [5] Medline: 99016060. Structure of an XRCC1 BRCT domain: a new protein-protein interaction module. Zhang X, Morera S, Bates PA, Whitehead PC, Coffey AI, Hainbuecher K, Nash RA, Sternberg MJ, Lindahl T, Freemont PS;

767. Kappa casein

15 Kappa-casein is a mammalian milk protein involved in a number of important physiological processes. In the gut, the ingested protein is split into an insoluble peptide (para kappa-casein) and a soluble hydrophilic glycopeptide (caseinomacropeptide). Caseinomacropeptide is responsible for increased efficiency of digestion, prevention of neonate hypersensitivity to ingested proteins, and inhibition of gastric pathogens. Number of members: 56

20 [1] Medline: 98072500. Nucleotide sequence evolution at the kappa-casein locus: evidence for positive selection within the family Bovidae. Ward TJ, Honeycutt RL, Derr JN; Genetics 1997;147:1863-1872.

768. Chitinases family 18 active site

25 PROSITE cross-reference(s) CHITINASE_18

Chitinases (EC 3.2.1.14) [1] are enzymes that catalyze the hydrolysis of the beta-1,4-N-acetyl-D-glucosamine linkages in chitin polymers. From the view point of sequence similarity chitinases belong to either family 18 or 19 in the classification of glycosyl hydrolases [2,E1]. Chitinases of family 18 (also known as classes III or V) groups a variety

30 of proteins:

a) Chitinases from:

- Prokaryotes such as Alteromonas, Bacillus, Serratia, Streptomyces, etc.
- Plants such as Arabidopsis, cucumber, bean, tobacco, etc.

- Fungi such as *Aphanocladium*, *Rhizopus*, *Saccharomyces*, etc.
- Nematode (*Brugia malayi*).
- Insects (*Manduca sexta*).
- Baculoviruses (*Autographa Californica* Nuclear Polyhedrosis virus).

5

b) Other proteins:

- Hevamine, a rubber tree protein with chitinase and lysozyme activities.
- *Cluyveromyces lactis* killer toxin alpha subunit, which acts as a chitinase.
- *Flavobacterium* and *Streptomyces* endo-beta-N-acetylglucosaminidases (EC 3.2.1.96).
- Mammalian di-N-acetylchitinase which is involved in the degradation of asparagine-linked glycoproteins.
- Human cartilage glycoprotein Gp-39.
- Jack bean concanavalin B (conB), a protein that has lost its catalytic activity.

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Site directed mutagenesis experiments [3] and crystallographic data [4,5] have shown that a conserved glutamate is involved in the catalytic mechanism and probably acts as a proton donor. This glutamate is at the extremity of the best conserved region in these proteins.

20

Consensus pattern[LIVMFY]-[DN]-G-[LIVMF]-[DN]-[LIVMF]-[DN]-x-E [E is the active site residue]

[1] Flach J., Pilet P.-E., Jolles P. *Experientia* 48:701-716(1992).

[2] Henrissat B. *Biochem. J.* 280:309-316(1991).

25

[3] Watanabe T., Kohori K., Miyashita K., Fujii T., Sakai H., Uchida M., Tanaka H. *J. Biol. Chem.* 268:18567-18572(1993).

[4] Perrakis A., Tews I., Dauter Z., Oppenheim A.B., Chet I., Wilson K.S., Vorgias C.E. *Structure* 2:1169-1180(1994).

[5] van Scheltinga A.C.T., Kalk K.H., Beintema J.J., Dijkstra B.W. *Structure* 2:1181-

30

1189(1994).

769. gag_p17. gag gene protein p17 (matrix protein).

The matrix protein forms an icosahedral shell associated with the inner membrane of the mature immunodeficiency virus. Number of members: 1598

[1] Medline: 95055757. Three-dimensional structure of the human immunodeficiency virus type 1 matrix protein. Massiah MA, Starich MR, Paschall C, Summers MF, Christensen AM, Sundquist WI; J Mol Biol 1994;244:198-223.

770. GDA1/CD39 family of nucleoside phosphatases signature

PROSITE cross-reference(s); GDA1_CD39_NTPASE

A number of nucleoside diphosphate and triphosphate hydrolases as well as some yet uncharacterized proteins have been found to belong to the same family [1, 2]. This family currently consist of:

- Yeast guanosine-diphosphatase (EC 3.6.1.42) (GDPase) (gene GDA1). GDA1 is a golgi integral membrane enzyme that catalyzes the hydrolysis of GDP to GMP.
- Potato apyrase (EC 3.6.1.5) (adenosine diphosphatase) (ADPase). Apyrase acts on both ATP and ADP to produce AMP.
- Mammalian vascular ATP-diphosphohydrolase (EC 3.6.1.5) (also known as lymphoid cell activation antigen CD39).
- Toxoplasma gondii nucleoside-triphosphatases (EC 3.6.1.15) (NTPase). NTPase hydrolyses various nucleoside triphosphates to produce the corresponding nucleoside mono- and diphosphates. This enzyme is secreted into the invaded host cell into the parasitophorous vacuole, a specialized compartment where the parasite intracellularly resides.
- Pea nucleoside-triphosphatases (EC 3.6.1.15) (NTPase).

- Caenorhabditis elegans hypothetical protein C33H5.14.

- Caenorhabditis elegans hypothetical protein R07E4.4.

- Yeast chromosome V hypothetical protein YER005w.

The above uncharacterized proteins all seem to be membrane-bound.

All these proteins share a number of conserved domains. The best conserved of these domains have been selected. It is located in the central section of the proteins.

Consensus pattern[LIVM]-x-G-x(2)-E-G-x-[FY]-x-[FW]-[LIVA]-[TAG]-x-N-[HY]

- [1] Handa M., Guidotti G. Biochem. Biophys. Res. Commun. 218:916-923(1996).
- [2] Vasconcelos E.G., Ferreira S.T., de Carvalho T.M.U., de Souza W., Kettlun A.M., Mancilla M., Valenzuela M.A., Verjovski-Almeida S. J. Biol. Chem. 271:22139-22145(1996).
771. GTP cyclohydrolase I signatures
 PROSITE cross-reference(s): GTP_CYCLOHYDROL_1_1, GTP_CYCLOHYDROL_1_2
 GTP cyclohydrolase I (EC 3.5.4.16) catalyzes the biosynthesis of formic acid and dihydroneopterin triphosphate from GTP. This reaction is the first step in the biosynthesis of tetrahydrofolate in prokaryotes, of tetrahydrobiopterin in vertebrates, and of pteridine-containing pigments in insects.
- GTP cyclohydrolase I is a protein of from 190 to 250 amino acid residues. The comparison of the sequence of the enzyme from bacterial and eukaryotic sources shows that the structure of this enzyme has been extremely well conserved throughout evolution [1].
- Two conserved regions were selected as signature patterns. The first contains a perfectly conserved tetrapeptide which is part of the GTP-binding pocket [2], the second region also contains conserved residues involved in GTP-binding.
- Consensus pattern[DEN]-[LIVM](2)-x(2)-[KRNQ]-[DEN]-[LIVM]-x(3)-[ST]-x-C-E- H-H
 Consensus pattern[SA]-x-[RK]-x-Q-[LIVM]-Q-E-[RN]-[LI]-[TSN]
- [1] Maier J., Witter K., Guetlich M., Ziegler I., Werner T., Ninnemann H. Biochem. Biophys. Res. Commun. 212:705-711(1995).
- [2] Nar H., Huber R., Meining W., Schmid C., Weinkauff S., Bacher A. Structure 3:459-466(1995).
772. IlvC. Acetohydroxy acid isomeroreductase
 Acetohydroxy acid isomeroreductase catalyses the conversion of acetohydroxy acids into dihydroxy valerates. This reaction is the second in the synthetic pathway of the essential branched side chain amino acids valine and isoleucine. Number of members: 29

[1] Medline: 97361822. The crystal structure of plant acetohydroxy acid isomeroreductase complexed with NADPH, two magnesium ions and a herbicidal transition state analog determined at 1.65 Å resolution. Biou V, Dumas R, Cohen-Addad C, Douce R, Job D, Pebay-Peyroula E; EMBO J 1997;16:3405-3415.

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773. Prokaryotic membrane lipoprotein lipid attachment site

PROSITE cross-reference(s); PROKAR_LIPOPROTEIN

In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which is cleaved by a specific lipoprotein signal peptidase (signal peptidase II). The peptidase recognizes a conserved sequence and cuts upstream of a cysteine residue to which a glyceride-fatty acid lipid is attached [1]. Some of the proteins known to undergo such processing currently include (for recent listings see [1,2,3]):

10

- Major outer membrane lipoprotein (murein-lipoproteins) (gene lpp).

- Escherichia coli lipoprotein-28 (gene nlpA).

15

- Escherichia coli lipoprotein-34 (gene nlpB).

- Escherichia coli lipoprotein nlpC.

- Escherichia coli lipoprotein nlpD.

- Escherichia coli osmotically inducible lipoprotein B (gene osmB).

- Escherichia coli osmotically inducible lipoprotein E (gene osmE).

20

- Escherichia coli peptidoglycan-associated lipoprotein (gene pal).

- Escherichia coli rare lipoproteins A and B (genes rplA and rplB).

- Escherichia coli copper homeostasis protein cutF (or nlpE).

- Escherichia coli plasmids traT proteins.

- Escherichia coli Col plasmids lysis proteins.

25

- A number of Bacillus beta-lactamases.

- Bacillus subtilis periplasmic oligopeptide-binding protein (gene oppA).

- Borrelia burgdorferi outer surface proteins A and B (genes ospA and ospB).

- Borrelia hermsii variable major protein 21 (gene vmp21) and 7 (gene vmp7).

- Chlamydia trachomatis outer membrane protein 3 (gene omp3).

30

- Fibrobacter succinogenes endoglucanase cel-3.

- Haemophilus influenzae proteins Pal and Pcp.

- Klebsiella pullulunase (gene pulA).

- Klebsiella pullulunase secretion protein pulS.

- Mycoplasma hyorhinis protein p37.

- Mycoplasma hyorhinis variant surface antigens A, B, and C (genes vlpABC).
- Neisseria outer membrane protein H.8.
- Pseudomonas aeruginosa lipopeptide (gene lppL).
- Pseudomonas solanacearum endoglucanase egl.
- 5 - Rhodopseudomonas viridis reaction center cytochrome subunit (gene cytC).
- Rickettsia 17 Kd antigen.
- Shigella flexneri invasion plasmid proteins mxiJ and mxiM.
- Streptococcus pneumoniae oligopeptide transport protein A (gene amiA).
- Treponema pallidum 34 Kd antigen.
- 10 - Treponema pallidum membrane protein A (gene tmpA).
- Vibrio harveyi chitobiase (gene chb).
- Yersinia virulence plasmid protein yscJ.

- Halocyanin from Natrobacterium pharaonis [4], a membrane associated copper-binding protein. This is the first archaeobacterial protein known to be modified in such a fashion).
- 15

From the precursor sequences of all these proteins, we derived a consensus pattern and a set of rules to identify this type of post-translational modification.

- 20 Consensus pattern{DERK}(6)-[LIVFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1) The cysteine must be between positions 15 and 35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the first seven positions of the sequence.

[1] Hayashi S., Wu H.C. J. Bioenerg. Biomembr. 22:451-471(1990).

25

[2] Klein P., Somorjai R.L., Lau P.C.K. Protein Eng. 2:15-20(1988).

[3] von Heijne G. Protein Eng. 2:531-534(1989).

[4] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

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774. Aminoacyl-transfer RNA synthetases class-II signatures

PROSITE cross-reference(s); AA_TRNA_LIGASE_II_1; AA_TRNA_LIGASE_II_2

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein

biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure.

The synthetases specific for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is different to the Rossmann fold observed for the class I synthetases [7].

Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions are present [2,5,8]. Signature patterns from two of these regions have been derived.

Consensus pattern[FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE]

Consensus pattern[GSTALVF]-{DENQHRKP}-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-[LIVMSTAG]-[LIVMFY]

[1]Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).

[2]Delarue M., Moras D. BioEssays 15:675-687(1993).

[3]Schimmel P. Trends Biochem. Sci. 16:1-3(1991).

[4]Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).

[5]Cusack S., Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991).

[6]Cusack S. Biochimie 75:1077-1081(1993).

[7]Cusack S., Berthet-Colominas C., Haertlein M., Nassar N., Leberman R. Nature 347:249-255(1990).

[8]Leveque F., Plateau P., Dessen P., Blanquet S. Nucleic Acids Res. 18:305-312(1990).

775. X. Trans-activation protein X

This protein is found in hepadnaviruses where it is indispensable for replication. Number of members: 91

776. Thymidylate synthase active site

Thymidylate synthase (EC 2.1.1.45) [1,2] catalyzes the reductive methylation of dUMP to dTMP with concomitant conversion of 5,10-methylenetetrahydrofolate to dihydrofolate. Thymidylate synthase plays an essential role in DNA synthesis and is an important target for certain chemotherapeutic drugs.

Thymidylate synthase is an enzyme of about 30 to 35 Kd in most species except in protozoan and plants where it exists as a bifunctional enzyme that includes a dihydrofolate reductase domain.

A cysteine residue is involved in the catalytic mechanism (it covalently binds the 5,6-dihydro-dUMP intermediate). The sequence around the active site of this enzyme is conserved from phages to vertebrates.

Consensus pattern R-x(2)-[LIVM]-x(3)-[FW]-[QN]-x(8,9)-[LV]-x-P-C-[HAVM]-x(3)-[QMT]-[FYW]-x-[LV] [C is the active site residue]

[1] Benkovic S.J. Annu. Rev. Biochem. 49:227-251(1980).

[2] Ross P., O'Gara F., Condon S. Appl. Environ. Microbiol. 56:2156-2163(1990).

777. Glycosyl hydrolases family 31 signatures

It has been shown [1,2,3,E1] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

- Lysosomal alpha-glucosidase (EC 3.2.1.20) (acid maltase) is a vertebrate glycosidase active at low pH, which hydrolyzes alpha(1->4) and alpha(1->6) linkages in glycogen, maltose, and isomaltose.

- Alpha-glucosidase (EC 3.2.1.20) from the yeast *Candida tsukunabaensis*.

- Alpha-glucosidase (EC 3.2.1.20) (gene *malA*) from the archaebacteria *Sulfolobus solfataricus*.

- Intestinal sucrase-isomaltase (EC 3.2.1.48 / EC 3.2.1.10) is a vertebrate membrane-bound, multifunctional enzyme complex which hydrolyzes sucrose, maltose and isomaltose. The sucrase and isomaltase domains of the enzyme are homologous (41% of amino acid identity) and have most probably evolved by duplication.

- Glucoamylase 1 (EC 3.2.1.3) (glucan 1,4-alpha-glucosidase) from various fungal species.

- Yeast hypothetical protein YBR229c.

- Fission yeast hypothetical protein SpAC30D11.01c.

An aspartic acid has been implicated [4] in the catalytic activity of sucrase, isomaltase, and lysosomal alpha-glucosidase. The region around this active residue is highly conserved and can be used as a signature pattern. A second region, which contains two conserved cysteines, has been used as an additional signature pattern.

5

Consensus pattern [GF]-[LIVMF]-W-x-D-M-[NSA]-E [D is the active site residue]

Consensus pattern G-[AV]-D-[LIVMTA]-C-G-[FY]-x(3)-[ST]-x(3)-L-C-x-R-W-x(2)-[LV]-[GSA]-[SA]-F-x-P-F-x-R-[DN]

10 [1] Henrissat B. Biochem. J. 280:309-316(1991).

[2] Kinsella B.T., Hogan S., Larkin A., Cantwell B.A. Eur. J. Biochem. 202:657-664(1991).

[3] Naim H.Y., Niermann T., Kleinhans U., Hollenberg C.P., Strasser A.W.M. FEBS Lett. 294:109-112(1991).

[4] Hermans M.M.P., Kroos M.A., van Beeumen J., Oostra B.A., Reuser A.J.J. J. Biol.

15 Chem. 266:13507-13512(1991).

778. Urease signatures

Urease (EC 3.5.1.5) is a nickel-binding enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia [1]. Historically, it was the first enzyme to be crystallized (in 20 1926). It is mainly found in plant seeds, microorganisms and invertebrates. In plants, urease is a hexamer of identical chains. In bacteria [2], it consists of either two or three different subunits (alpha, beta and gamma).

Urease binds two nickel ions per subunit; four histidine, an aspartate and a carbamated-lysine serve as ligands to these metals; an additional histidine is involved in the 25 catalytic mechanism [3].

As signatures for this enzyme, a region was selected that contains two histidine that bind one of the nickel ions and the region of the active site histidine.

30

Consensus pattern T-[AY]-[GA]-[GAT]-[LIVM]-D-x-H-[LIVM]-H-x(3)-P [The two H's bind nickel]

Consensus pattern [LIVM](2)-[CT]-H-[HN]-L-x(3)-[LIVM]-x(2)-D-[LIVM]-x-F-A [H is the active site residue]

[1] Takishima K., Suga T., Mamiya G. Eur. J. Biochem. 175:151-165(1988).

[2] Mobley H.L.T., Husinger R.P. Microbiol. Rev. 53:85-108(1989).

[3] Jabri E., Carr M.B., Hausinger R.P., Karplus P.A. Science 268:998-1004(1995).

779. Tyrosine specific protein phosphatases signature and profiles

- 5 Tyrosine specific protein phosphatases (EC 3.1.3.48) (PTPase) [1 to 5] are enzymes that catalyze the removal of a phosphate group attached to a tyrosine residue. These enzymes are very important in the control of cell growth, proliferation, differentiation and transformation. Multiple forms of PTPase have been characterized and can be classified into two categories: soluble PTPases and transmembrane receptor proteins that contain PTPase
- 10 domain(s). The currently known PTPases are listed below:

Soluble PTPases.

- PTPN1 (PTP-1B).
- PTPN2 (T-cell PTPase; TC-PTP).
- 15 - PTPN3 (H1) and PTPN4 (MEG), enzymes that contain an N-terminal band 4.1- like domain (see <PDOC00566>) and could act at junctions between the membrane and cytoskeleton.
- PTPN5 (STEP).
- PTPN6 (PTP-1C; HCP; SHP) and PTPN11 (PTP-2C; SH-PTP3; Syp), enzymes which
- 20 contain two copies of the SH2 domain at its N-terminal extremity. The Drosophila protein corkscrew (gene csw) also belongs to this subgroup.
- PTPN7 (LC-PTP; Hematopoietic protein-tyrosine phosphatase; HePTP).
- PTPN8 (70Z-PEP).
- PTPN9 (MEG2).
- 25 - PTPN12 (PTP-G1; PTP-P19).
- Yeast PTP1.
- Yeast PTP2 which may be involved in the ubiquitin-mediated protein degradation pathway.
- Fission yeast pyp1 and pyp2 which play a role in inhibiting the onset of mitosis.
- 30 - Fission yeast pyp3 which contributes to the dephosphorylation of cdc2.
- Yeast CDC14 which may be involved in chromosome segregation.
- Yersinia virulence plasmid PTPases (gene yopH).
- Autographa californica nuclear polyhedrosis virus 19 Kd PTPase.

Dual specificity PTPases.

- DUSP1 (PTPN10; MAP kinase phosphatase-1; MKP-1); which dephosphorylates MAP kinase on both Thr-183 and Tyr-185.
- DUSP2 (PAC-1), a nuclear enzyme that dephosphorylates MAP kinases ERK1 and ERK2 on both Thr and Tyr residues.
- DUSP3 (VHR).
- DUSP4 (HVH2).
- DUSP5 (HVH3).
- DUSP6 (Pyst1; MKP-3).
- DUSP7 (Pyst2; MKP-X).
- Yeast MSG5, a PTPase that dephosphorylates MAP kinase FUS3.
- Yeast YVH1.
- Vaccinia virus H1 PTPase; a dual specificity phosphatase.

15 Receptor PTPases.

Structurally, all known receptor PTPases, are made up of a variable length extracellular domain, followed by a transmembrane region and a C-terminal catalytic cytoplasmic domain. Some of the receptor PTPases contain fibronectin type III (FN-III) repeats, immunoglobulin-like domains, MAM domains or carbonic anhydrase-like domains in their extracellular region. The cytoplasmic region generally contains two copies of the PTPase domain. The first seems to have enzymatic activity, while the second is inactive but seems to affect substrate specificity of the first. In these domains, the catalytic cysteine is generally conserved but some other, presumably important, residues are not.

25 In the following table, the domain structure of known receptor PTPases is shown:

Extracellular	Intracellular			
-----	-----	-----	-----	-----
Ig	FN-3	CAH	MAM	PTPase

Leukocyte common antigen (LCA) (CD45)	0	2	0	0	2
Leukocyte antigen related (LAR)	3	8	0	0	2
Drosophila DLAR	3	9	0	0	2
Drosophila DPTP	2	2	0	0	2

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	PTP-alpha (LRP)	0	0	0	0	2
	PTP-beta	0	16	0	0	1
	PTP-gamma	0	1	1	0	2
	PTP-delta	0	>7	0	0	2
5	PTP-epsilon	0	0	0	0	2
	PTP-kappa	1	4	0	1	2
	PTP-mu	1	4	0	1	2
	PTP-zeta	0	1	1	0	2

10 PTPase domains consist of about 300 amino acids. There are two conserved cysteines, the second one has been shown to be absolutely required for activity. Furthermore, a number of conserved residues in its immediate vicinity have also been shown to be important.

A signature pattern was derived for PTPase domains centered on the active site cysteine.

15 There are three profiles for PTPases, the first one spans the complete domain and is not specific to any subtype. The second profile is specific to dual-specificity PTPases and the third one to the PTP subfamily.

Consensus pattern [LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAGP]-x-[LIVMFY] [C is the active site residue]

20 Note the M-phase inducer phosphatases (cdc25-type phosphatase) are tyrosine- protein phosphatases that are not structurally related to the above PTPases.

Note this documentation entry is linked to both a signature pattern and to profiles. As profiles are much more sensitive than the pattern, you should use them if you have access to the necessary software tools to do so.

25

- [1] Fischer E.H., Charbonneau H., Tonks N.K. Science 253:401-406(1991).
- [2] Charbonneau H., Tonks N.K. Annu. Rev. Cell Biol. 8:463-493(1992).
- [3] Trowbridge I.S. J. Biol. Chem. 266:23517-23520(1991).
- [4] Tonks N.K., Charbonneau H. Trends Biochem. Sci. 14:497-500(1989).
- 30 [5] Hunter T. Cell 58:1013-1016(1989).

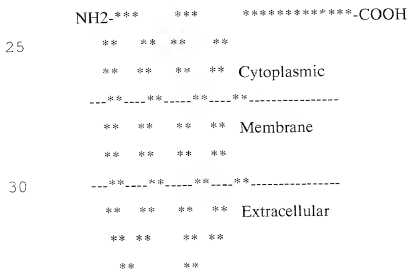
780. Connexins signatures

Gap junctions [1] are specialized regions of the plasma membrane which consist of closely packed pairs of transmembrane channels, the connexons, through which small

molecules diffuse from a cell to a neighboring cell. Each connexon is composed of an hexamer of an integral membrane protein which is often referred to as connexin. In a given species there are a number of different, yet structurally related, tissue specific, forms of connexins. The types of connexins which are currently known are listed below.

- 5 - Connexin 56 (Cx56).
- Connexin 50 (Cx50) (lens fiber protein MP70).
- Connexin 46 (Cx46) (alpha-3).
- Connexin 45 (Cx45) (alpha-6).
- Connexin 43 (Cx43) (alpha-1).
- 10 - Connexin 40 (Cx40) (alpha-5).
- Connexin 38 (Cx38) (alpha-2).
- Connexin 37 (Cx37) (alpha-4).
- Connexin 33 (Cx33) (alpha-7).
- Connexin 32 (Cx32) (beta-1).
- 15 - Connexin 31.1 (Cx31.1) (beta-4).
- Connexin 31 (Cx31) (beta-3).
- Connexin 30.3 (Cx30.3) (beta-5).
- Connexin 26 (Cx26) (beta-2).

Structurally the connexins consist of a short cytoplasmic N-terminal domain, followed by four transmembrane segments that delimit two extracellular and one cytoplasmic loops; the C-terminal domain is cytoplasmic and its length is variable (from 20 residues in Cx26 to 260 residues in Cx56). The schematic representation of this structure is shown below.



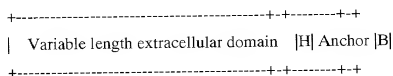
The sequences of the two extracellular loops are well conserved. In both loops there are three conserved cysteines which are involved in disulfide bonds. A signature patterns from each of these two loop regions has been built.

- 5 Consensus patternC-[DN]-T-x-Q-P-G-C-x(2)-V-C-[FY]-D [The three C's are involved in
disulfide bonds] Consensus patternC-x(3,4)-P-C-x(3)-[LIVM]-[DEN]-C-[FY]-[LIVM]-[SA]-
[KR]-P [The three C's are involved in disulfide bonds]

[1] Goodenough D.A., Goliger J.A., Paul D.L. Annu. Rev. Biochem. 65:475-502(1996).

781. Gram-positive cocci surface proteins 'anchoring' hexapeptide

Surface proteins from Gram-positive cocci contains a conserved hexapeptide located a few residues downstream of a hydrophobic C-terminal membrane anchor region which is followed by a cluster of basic amino acids [1]. This structure is represented in the following schematic representation:



20 'H': conserved hexapeptide.

'B': cluster of basic residues.

It has been proposed that this hexapeptide sequence is responsible for a post-translational modification necessary for the proper anchoring of the proteins which bear it, to the cell wall.

- 25 Proteins known to contain such hexapeptide are listed below:
- Aggregation substance from streptococcus faecalis (asaI).
 - C5a peptidase from Streptococcus pyogenes (scpA).
 - C protein alpha-antigen from Streptococcus agalactiae (bca).
 - Cell surface antigen I/II (PAC) from Streptococcus mutans.
 - 30 - Dextranase from Streptococcus downei (dex).
 - Fibronectin-binding protein from Staphylococcus aureus (fnbA).
 - Fimbrial subunits from Actinomyces naeslundii and viscosus.
 - IgA binding protein from Streptococcus pyogenes (arp4).
 - IgA binding protein (B antigen) from Streptococcus agalactiae (bag).

- IgG binding proteins from *Streptococci* and *Staphylococcus aureus*.
- Internalin A from *Listeria monocytogenes* (inlA).
- M proteins from streptococci.
- Muramidase-released protein from *Streptococcus suis* (mrp).
- 5 - Nisin leader peptide processing protease from *Lactococcus lactis* (nisP).
- Protein A from *Staphylococcus aureus*.
- Trypsin-resistant surface T protein from streptococci.
- Wall-associated protein from *Streptococcus mutans* (wapA).
- Wall-associated serine proteinases from *Lactococcus lactis*.

10

Consensus pattern L-P-x-T-G-[STGAVDE]

[1] Schneewind O., Jones K.F., Fischetti V.A. J. Bacteriol. 172:3310-3317(1990).

15 782. Gamma-glutamyltranspeptidase signature

Gamma-glutamyltranspeptidase (EC 2.3.2.2) (GGT) [1] catalyzes the transfer of the gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate). GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione. In prokaryotes and eukaryotes, it is an enzyme that consists of two polypeptide chains, a heavy and a light subunit, processed from a single chain precursor. The active site of GGT is known to be located in the light subunit.

The sequences of mammalian and bacterial GGT show a number of regions of high similarity [2]. *Pseudomonas cephalosporin acylases* (EC 3.5.1.-) that convert 7-beta-(4-carboxybutanamido)-cephalosporanic acid (GL-7ACA) into 7-aminocephalosporanic acid (7ACA) and glutaric acid are evolutionary related to GGT and also show some GGT activity [3]. Like GGT, these GL-7ACA acylases, are also composed of two subunits.

One of the conserved regions correspond to the N-terminal extremity of the mature light chains of these enzymes. This region has been used as a signature pattern.

30 Consensus pattern T-[STA]-H-x-[ST]-[LIVMA]-x(4)-G-[SN]-x-V-[STA]-x-T-x-T-[LIVM]-[NE]-x(1,2)-[FY]-G

[1] Tate S.S., Meister A. Meth. Enzymol. 113:400-419(1985).

[2] Suzuki H., Kumagai H., Echigo T., Tochikura T. J. Bacteriol. 171:5169-5172(1989).

[3] Ishiye M., Niwa M. Biochim. Biophys. Acta 1132:253-239(1992).

783. Ferrochelatase signature

5 Ferrochelatase (EC 4.99.1.1) (protoheme ferro-lyase) [1,2] catalyzes the last step in heme biosynthesis: the chelation of a ferrous ion to proto-porphyrin IX, to form protoheme.

In eukaryotes, ferrochelatase is a mitochondrial protein bound to the inner membrane, whose active site faces the mitochondrial matrix. The mature form of eukaryotic ferrochelatase is composed of about 360 amino acids. In bacteria, ferrochelatase (gene hemH) [3] is a protein of from 310 to 380 amino acids.

10 The human autosomal dominant disease protoporphyria is due to the reduced activity of ferrochelatase.

The signature pattern for this enzyme is based on a conserved region which contains a histidine residue which could be involved in binding iron.

15 Consensus pattern[LIVMF](2)-x-[ST]-x-H-[GS]-[LIVM]-P-x(4,5)-[DENQKR]-x-G-[DP]-x(1,2)-Y

[1] Labbe-Bois R. J. Biol. Chem. 265:7278-7283(1990).

[2] Brenner D.A., Frasier F. Proc. Natl. Acad. Sci. U.S.A. 88:849-853(1991).

20 [3] Miyamoto K., Nakahigashi K., Nishimura K., Inokuchi H. J. Mol. Biol. 219:393-398(1991).

784. Cellulose-binding domain, bacterial type

25 The microbial degradation of cellulose and xylans requires several types of enzyme such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1].

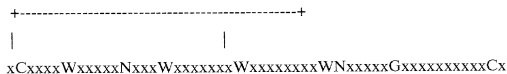
Structurally, cellulases and xylanases generally consist of a catalytic domain joined to a cellulose-binding domain (CBD) by a short linker sequence rich in proline and/or hydroxy-amino acids.

30 The CBD of a number of bacterial cellulases has been shown to consist of about 105 amino acid residues [2]. Enzymes known to contain such a domain are:

- Endoglucanase (gene end1) from *Butyrivibrio fibrisolvens*.
- Endoglucanases A (gene cenA) and B (cenB) from *Cellulomonas fimi*.
- Exoglucanases A (gene cbhA) and B (cbhB) from *Cellulomonas fimi*.

- Endoglucanase E-2 (gene celB) from *Thermomonospora fusca*.
- Endoglucanase A (gene celA) from *Microbispora bispora*.
- Endoglucanases A (gene celA), B (celB) and C (celC) from *Pseudomonas fluorescens*.
- Endoglucanase A (gene celA) from *Streptomyces lividans*.
- 5 - Exocellobiohydrolase (gene cex) from *Cellulomonas fimi*.
- Xylanases A (gene xynA) and B (xynB) from *Pseudomonas fluorescens*.
- Arabinofuranosidase C (EC 3.2.1.55) (xylanase C) (gene xynC) from *Pseudomonas fluorescens*.
- Chitinase 63 (EC 3.2.1.14) from *Streptomyces plicatus*.
- 10 - Chitinase C from *Streptomyces lividans*.

The CBD domain is found either at the N-terminal or at the C-terminal extremity of these enzymes. As it is shown in the following schematic representation, there are two conserved cysteines in this CBD domain - one at each extremity of the domain - which have been shown [3] to be involved in a disulfide bond. There are also four conserved tryptophan residues which could be involved in the interaction of the CBD with polysaccharides.



'C': conserved cysteine involved in a disulfide bond. '*': position of the pattern.

Consensus pattern W-N-[STAGR]-[STDN]-[LIVM]-x(2)-[GST]-x-[GST]-x(2)-[LIVMFT]-[GA]

- [1] Gilkes N.R., Hennissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).
- [2] Meinke A., Gilkes N.R., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Protein Seq. Data Anal. 4:349-353(1991).
- [3] Gilkes N.R., Claeysens M., Aebersold R., Hennissat B., Meinke A., Morrison H.D., Kilburn D.G., Warren R.A.J., Miller R.C. Jr. Eur. J. Biochem. 202:367-377(1991).

785. Amidases signature

It has been shown [1,2,3] that several enzymes from various prokaryotic and eukaryotic organisms which are involved in the hydrolysis of amides (amidases) are evolutionary related. These enzymes are listed below.

- Indoleacetamide hydrolase (EC 3.5.1.-), a bacterial plasmid-encoded enzyme that catalyzes the hydrolysis of indole-3-acetamide (IAM) into indole-3-acetate (IAA), the second step in the biosynthesis of auxins from tryptophan.
- Acetamidase from *Emericella nidulans* (gene *amdS*), an enzyme which allows acetamide to be used as a sole carbon or nitrogen source.
- Amidase (EC 3.5.1.4) from *Rhodococcus* sp. N-774 and *Brevibacterium* sp. R312 (gene *amdA*). This enzyme hydrolyzes propionamides efficiently, and also at a lower efficiency, acetamide, acrylamide and indoleacetamide.
- Amidase (EC 3.5.1.4) from *Pseudomonas chlororaphis*.
- 6-aminohexanoate-cyclic-dimer hydrolase (EC 3.5.2.12) (nylon oligomers degrading enzyme E1) (gene *nylA*), a bacterial plasmid encoded enzyme which catalyzes the first step in the degradation of 6-aminohexanoic acid cyclic dimer, a by-product of nylon manufacture [4].
- Glutamyl-tRNA(Gln) amidotransferase subunit A [5].
- Mammalian fatty acid amide hydrolase (gene *FAAH*) [6].
- A putative amidase from yeast (gene *AMD2*).
- *Mycobacterium tuberculosis* putative amidases *amiA2*, *amiB2*, *amiC* and *amiD*.

All these enzymes contain in their central section a highly conserved region rich in glycine, serine, and alanine residues. This region has been used as a signature pattern.

- Consensus pattern: G-[GA]-S-[GS]-[GS]-G-x-[GSA]-[GSAVY]-x-[LIVM]-[GSA]-x(6)-[GSAT]-x-[GA]-x-[DE]-x-[GA]-x-S-[LIVM]-R-x-P-[GSAC]

[1] Mayaux J.-F., Cerbelaud E., Soubrier F., Faucher D., Petre D. J. *Bacteriol.* 172:6764-6773(1990).

[2] Hashimoto Y., Nishiyama M., Ikehata O., Horinouchi S., Beppu T. *Biochim. Biophys. Acta* 1088:225-233(1991).

[3] Chang T.-H., Abelson J. *Nucleic Acids Res.* 18:7180-7180(1990).

[4] Tsuchiya K., Fukuyama S., Kanzaki N., Kanagawa K., Negoro S., Okada H. J. *Bacteriol.* 171:3187-3191(1989).

[5] Curnow A.W., Hong K.W., Yuan R., Kim S.I., Martins O., Winkler W., Henkin T.M., Soll D. Proc. Natl. Acad. Sci. U.S.A. 94:11819-11826(1997).

[6] Cravatt B.F., Giang D.K., Mayfield S.P., Boger D.L., Lerner R.A., Gilula N.B. Nature 384:83-87(1996).

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786. Glycosyl hydrolases family 10 active site

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1,2]. Fungi and bacteria produces a spectrum of cellulolytic enzymes (cellulases) and xylanases which, on the basis of sequence similarities, can be classified into families. One of these families is known as the cellulase family F [3] or as the glycosyl hydrolases family 10 [4,E1]. The enzymes which are currently known to belong to this family are listed below.

10

- *Aspergillus awamori* xylanase A (xynA).

15

- *Bacillus* sp. strain 125 xylanase (xynA).

- *Bacillus stearothermophilus* xylanase.

- *Butyrivibrio fibrisolvens* xylanases A (xynA) and B (xynB).

- *Caldocellum saccharolyticum* bifunctional endoglucanase/exoglucanase (celB). This protein consists of two domains; it is the N-terminal domain, which has exoglucanase activity, which belongs to this family.

20

- *Caldocellum saccharolyticum* xylanase A (xynA).

- *Caldocellum saccharolyticum* ORF4. This hypothetical protein is encoded in the xynABC operon and is probably a xylanase.

- *Cellulomonas fimi* exoglucanase/xylanase (cex).

25

- *Clostridium stercorarium* thermostable cellobiohydrolase.

- *Clostridium thermocellum* xylanases Y (xynY) and Z (xynZ).

- *Cryptococcus albidus* xylanase.

- *Penicillium chrysogenum* xylanase (gene xylP).

- *Pseudomonas fluorescens* xylanases A (xynA) and B (xynB).

30

- *Ruminococcus flavefaciens* bifunctional xylanase XYLA (xynA). This protein consists of three domains: a N-terminal xylanase catalytic domain that belongs to family 11 of glycosyl hydrolases; a central domain composed of short repeats of Gln, Asn and Trp, and a C-terminal xylanase catalytic domain that belongs to family 10 of glycosyl hydrolases.

- *Streptomyces lividans* xylanase A (xlnA).

- *Thermoanaerobacter saccharolyticum* endoxylanase A (xynA).
- *Thermoascus aurantiacus* xylanase.
- *Thermophilic bacterium* Rt8.B4 xylanase (xynA).

5 One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [5], in the exoglucanase from *Cellulomonas fimi*, to be directly involved in glycosidic bond cleavage by acting as a nucleophile. This region has been used as a signature pattern.

10 Consensus pattern[GTA]-x(2)-[LIVN]-x-[IVMF]-[ST]-E-[LIY]-[DN]-[LIVMF] [E is the active site residue]

[1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).

[2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).

[3] Henrissat B., Claeysens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).

[4] Henrissat B. Biochem. J. 280:309-316(1991).

[5] Tull D., Withers S.G., Gilkes N.R., Kilburn D.G., Warren R.A.J., Aebersold R. J. Biol. Chem. 266:15621-15625(1991).

20 787. Fructose-bisphosphate aldolase class-II signatures

Fructose-bisphosphate aldolase (EC 4.1.2.13) [1,2] is a glycolytic enzyme that catalyzes the reversible aldol cleavage or condensation of fructose-1,6- bisphosphate into dihydroxyacetone-phosphate and glyceraldehyde 3-phosphate. There are two classes of fructose-bisphosphate aldolases with different catalytic mechanisms. Class-II aldolases [2], mainly found in prokaryotes and fungi, are homodimeric enzymes which require a divalent metal ion - generally zinc - for their activity.

This family also includes the following proteins:

- *Escherichia coli* galactitol operon protein gatY which catalyzes the transformation of tagatose 1,6-bisphosphate into glycerone phosphate and D- glyceraldehyde 3-phosphate.
- *Escherichia coli* N-acetyl galactosamine operon protein agaY which catalyzes the same reaction as that of gatY.

As signature patterns for this class of enzyme, two conserved regions were selected. The first pattern is located in the first half of the sequence and contains two histidine residues that have been shown [4] to be involved in binding a zinc ion. The second is located in the C-terminal section and contains clustered acidic residues and glycines.

5

Consensus pattern[FYVMT]-x(1,3)-[LIVMH]-[APN]-[LIVM]-x(1,2)-[LIVM]-H-x-D-H-[GACH] [The two H's are zinc ligands]

Consensus pattern[LIVM]-E-x-E-[LIVM]-G-x(2)-[GM]-[GSTA]-x-E

10 [1] Perham R.N. Biochem. Soc. Trans. 18:185-187(1990).

[2] Marsh J.J., Lebherz H.G. Trends Biochem. Sci. 17:110-113(1992).

[3] von der Osten C.H., Barbas C.F. III, Wong C.-H., Sinskey A.J. Mol. Microbiol. 3:1625-1637(1989).

[4] Berry A., Marshall K.E. FEBS Lett. 318:11-16(1993).

15

788. Prolyl oligopeptidase family serine active site

The prolyl oligopeptidase family [1,2,3] consist of a number of evolutionary related peptidases whose catalytic activity seems to be provided by a charge relay system similar to that of the trypsin family of serine proteases, but which evolved by independent convergent evolution. The known members of this family are listed below.

20

- Prolyl endopeptidase (EC 3.4.21.26) (PE) (also called post-proline cleaving enzyme). PE is an enzyme that cleaves peptide bonds on the C-terminal side of prolyl residues. The sequence of PE has been obtained from a mammalian species (pig) and from bacteria (*Flavobacterium meningosepticum* and *Aeromonas hydrophila*); there is a high degree of sequence

25

conservation between these sequences.

- *Escherichia coli* protease II (EC 3.4.21.83) (oligopeptidase B) (gene prtB) which cleaves peptide bonds on the C-terminal side of lysyl and arginyl residues.

- Dipeptidyl peptidase IV (EC 3.4.14.5) (DPP IV). DPP IV is an enzyme that removes N-terminal dipeptides sequentially from polypeptides having unsubstituted N-termini provided that the penultimate residue is proline.

30

- Yeast vacuolar dipeptidyl aminopeptidase A (DPAP A) (gene: STE13) which is responsible for the proteolytic maturation of the alpha-factor precursor.

- Yeast vacuolar dipeptidyl aminopeptidase B (DPAP B) (gene: DAP2).

- Acylamino-acid-releasing enzyme (EC 3.4.19.1) (acyl-peptide hydrolase). This enzyme catalyzes the hydrolysis of the amino-terminal peptide bond of an N-acetylated protein to generate a N-acetylated amino acid and a protein with a free amino-terminus.

- 5 A conserved serine residue has experimentally been shown (in E.coli protease II as well as in pig and bacterial PE) to be necessary for the catalytic mechanism. This serine, which is part of the catalytic triad (Ser, His, Asp), is generally located about 150 residues away from the C-terminal extremity of these enzymes (which are all proteins that contains about 700 to 800 amino acids).

10 Consensus pattern D-x(3)-A-x(3)-[LIVMFYW]-x(14)-G-x-S-x-G-G-[LIVMFYW](2) [S is the active site residue]

Note these proteins belong to families S9A/S9B/S9C in the classification of peptidases [4,E1].

[1] Rawlings N.D., Polgar L., Barrett A.J. Biochem. J. 279:907-911(1991).

[2] Barrett A.J., Rawlings N.D. Biol. Chem. Hoppe-Seyler 373:353-360(1992).

[3] Polgar L., Szabo E.

20 Biol. Chem. Hoppe-Seyler 373:361-366(1992).

[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

789. Formate--tetrahydrofolate ligase signatures

Formate--tetrahydrofolate ligase (EC 6.3.4.3) (formyltetrahydrofolate synthetase)

25 (FTHFS) is one of the enzymes participating in the transfer of one-carbon units, an essential element of various biosynthetic pathways. In many of these processes the transfers of one-carbon units are mediated by the coenzyme tetrahydrofolate (THF). Various reactions generate one-carbon derivatives of THF which can be interconverted between different oxidation states by FTHFS, methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5) and methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9).

30 In eukaryotes the FTHFS activity is expressed by a multifunctional enzyme, C-1-tetrahydrofolate synthase (C1-THF synthase), which also catalyzes the dehydrogenase and cyclohydrolase activities. Two forms of C1-THF synthases are known [1], one is located in the mitochondrial matrix, while the second one is cytoplasmic. In both forms the FTHFS

domain consist of about 600 amino acid residues and is located in the C-terminal section of C1-THF synthase. In prokaryotes FTHFS activity is expressed by a monofunctional homotetrameric enzyme of about 560 amino acid residues [2].

The sequence of FTHFS is highly conserved in all forms of the enzyme. As signature patterns, two regions that are almost perfectly conserved were selected. The first one is a glycine-rich segment located in the N-terminal part of FTHFS and which could be part of an ATP-binding domain [2]. The second pattern is located in the central section of FTHFS.

Consensus pattern G-[LIVM]-K-G-G-A-A-G-G-G-Y

Consensus pattern V-A-T-[IV]-R-A-L-K-x-[HN]-G-G

[1] Shannon K.W., Rabinowitz J.C. J. Biol. Chem. 263:7717-7725(1988).

[2] Lovell C.R., Przybyla A., Ljungdahl L.G. Biochemistry 29:5687-5694(1990).

790. Transthyretin signatures

Transthyretin (prealbumin) [1] is a thyroid hormone-binding protein that seems to transport thyroxine (T4) from the bloodstream to the brain. It is a protein of about 130 amino acids that assembles as a homotetramer and forms an internal channel that binds thyroxine. Transthyretin is mainly synthesized in the brain choroid plexus. In humans, variants of the protein are associated with distinct forms of amyloidosis.

The sequence of transthyretin is highly conserved in vertebrates. A number of uncharacterized proteins also belong to this family:

- Escherichia coli hypothetical protein yedX.
- Bacillus subtilis hypothetical protein yunM.
- Caenorhabditis elegans hypothetical protein R09H10.3.
- Caenorhabditis elegans hypothetical protein ZK697.8.

Two regions were selected as signature patterns. The first located in the N-terminal extremity starts with a lysine known to be involved in binding T4. The second pattern is located in the C-terminal extremity.

Consensus pattern [KH]-[IV]-L-[DN]-x(3)-G-x-P-A-x(2)-[IV]-x-[IV] [The K binds thyroxine]

Consensus pattern Y-[TH]-[IV]-[AP]-x(2)-L-S-[PQ]-[FYW]-[GS]-[FY]-[QS]

[1] Schreiber G., Richardson S.J. Comp. Biochem. Physiol. 116B:137-160(1997).

791. Dihydropteroate synthase signatures

All organisms require reduced folate cofactors for the synthesis of a variety of metabolites. Most microorganisms must synthesize folate de novo because they lack the active transport system of higher vertebrate cells which allows these organisms to use dietary folates. Enzymes that are involved in the biosynthesis of folates are therefore the target of a variety of antimicrobial agents such as trimethoprim or sulfonamides.

Dihydropteroate synthase (EC 2.5.1.15) (DHPS) catalyzes the condensation of 6-hydroxymethyl-7,8-dihydropteridine pyrophosphate to para-aminobenzoic acid to form 7,8-dihydropteroate. This is the second step in the three steps pathway leading from 6-hydroxymethyl-7,8-dihydropterin to 7,8-dihydrofolate. DHPS is the target of sulfonamides which are substrates analog that compete with para-aminobenzoic acid.

Bacterial DHPS (gene *sul* or *folP*) [1] is a protein of about 275 to 315 amino acid residues which is either chromosomally encoded or found on various antibiotic resistance plasmids. In the lower eukaryote *Pneumocystis carinii*, DHPS is the C-terminal domain of a multifunctional folate synthesis enzyme (gene *fas*) [2].

Two signature patterns for DHPS were developed, the first signature is located in the N-terminal section of these enzymes, while the second signature is located in the central section.

Consensus pattern[LIVM]-x-[AG]-[LIVMF](2)-N-x-T-x-D-S-F-x-D-x-[SG]

Consensus pattern[GE]-[SA]-x-[LIVM](2)-D-[LIVM]-G-[GP]-x(2)-[STA]-x-P

[1] Slock J., Stahly D.P., Han C.-Y., Six E.W., Crawford I.P. J. Bacteriol. 172:7211-7226(1990).

[2] Volpes F., Dyer M., Scaife J.G., Darby G., Stammers D.K., Delves C.J. Gene 112:213-218(1992).

792. Phosphatidylinositol 3- and 4-kinases signatures

Phosphatidylinositol 3-kinase (PI3-kinase) (EC 2.7.1.137) [1] is an enzyme that phosphorylates phosphoinositides on the 3-hydroxyl group of the inositol ring. The exact function of the three products of PI3-kinase - PI-3-P, PI-3,4-P(2) and PI-3,4,5-P(3) - is not

yet known, although it is proposed that they function as second messengers in cell signalling. Currently, three forms of PI3-kinase are known:

- The mammalian enzyme which is a heterodimer of a 110 Kd catalytic chain (p110) and an 85 Kd subunit (p85) which allows it to bind to activated tyrosine protein kinases. There are at

5 least two different types of p100 subunits (alpha and beta).

- Yeast TOR1/DDR1 and TOR2/DDR2 [2], PI3-kinases required for cell cycle activation.

Both are proteins of about 280 Kd.

- Yeast VPS34 [3], a PI3-kinase involved in vacuolar sorting and segregation. VPS34 is a protein of about 100 Kd.

10 - Arabidopsis thaliana and soybean VPS34 homologs.

Phosphatidylinositol 4-kinase (PI4-kinase) (EC 2.7.1.67) [4] is an enzyme that acts on phosphatidylinositol (PI) in the first committed step in the production of the second messenger inositol-1,4,5,-trisphosphate. Currently the following forms of PI4-kinases are

15 known:

- Human PI4-kinase alpha.

- Yeast PIK1, a nuclear protein of 120 Kd.

- Yeast STT4, a protein of 214 Kd.

20 The PI3- and PI4-kinases share a well conserved domain at their C-terminal section; this domain seems to be distantly related to the catalytic domain of protein kinases [2]. Two signature patterns were developed from the best conserved parts of this domain.

Four additional proteins belong to this family:

25 - Mammalian FKBP-rapamycin associated protein (FRAP) [5], which acts as the target for the cell-cycle arrest and immunosuppressive effects of the FKBP12-rapamycin complex.

- Yeast protein ESR1 [6] which is required for cell growth, DNA repair and meiotic recombination.

- Yeast protein TEL1 which is involved in controlling telomere length.

30 - Yeast hypothetical protein YHR099w, a distantly related member of this family.

- Fission yeast hypothetical protein SpAC22E12.16C.

Consensus pattern[LIVMFAC]-K-x(1,3)-[DEA]-[DE]-[LIVMC]-R-Q-[DE]-x(4)-Q

Consensus pattern[GS]-x-[AV]-x(3)-[LIVM]-x(2)-[FYH]-[LIVM](2)-x-[LIVMF]-x-D-R-H-x(2)-N

[1] Hiles I.D., Otsu M., Volinia S., Fry M.J., Gout I., Dhand R., Panayotou G., Ruiz-Larrea F., Thompson A., Totty N.F., Hsuan J.J., Courtneidge S.A., Parker P.J., Waterfield M.D. Cell 70:419-429(1992).

[2] Kunz J., Henriquez R., Schneider U., Deuter-Reinhard M., Movva N., Hall M.N. Cell 73:585-596(1993).

[3] Schu P.V., Takegawa K., Fry M.J., Stack J.H., Waterfield M.D., Emr S.D. Science 260:88-91(1993).

[4] Garcia-Bustos J.F., Marini F., Stevenson I., Frei C., Hall M.N. EMBO J. 13:2352-2361(1994).

[5] Brown E.J., Albers M.W., Shin T.B., Ichikawa K., Keith C.T., Lane W.S., Schreiber S.L. Nature 369:756-758(1994).

[6] Kato R., Ogawa H. Nucleic Acids Res. 22:3104-3112(1994).

793. FAD-dependent glycerol-3-phosphate dehydrogenase signatures

FAD-dependent glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) (GPD) catalyzes the conversion of glycerol-3-phosphate into dihydroxyacetone phosphate. In bacteria [1] it is associated with the utilization of glycerol coupled to respiration. In *Escherichia coli*, two isozymes are known: one expressed under anaerobic conditions (gene *glpA*) and one in aerobic conditions (gene *glpD*). In eukaryotes, a mitochondrial form of GPD participates in the glycerol phosphate shuttle in conjunction with an NAD-dependent cytoplasmic GPD (EC 1.1.1.8) [2,3].

These enzymes are proteins of about 60 to 70 Kd which contain a probable FAD-binding domain in their N-terminal extremity. The mammalian enzyme differs from the bacterial or yeast proteins by having an EF-hand calcium-binding region (See <PDOC00018>) in its C-terminal extremity.

Two signature patterns were developed. One based on the first half of the FAD-binding domain and one which corresponds to a conserved region in the central part of these enzymes.

Consensus pattern[IV]-G-G-G-x(2)-G-[STACV]-G-x-A-x-D-x(3)-R-G

Consensus patternG-G-K-x(2)-[GSTE]-Y-R-x(2)-A

[1] Austin D., Larson T.J. J. Bacteriol. 173:101-107(1991).

[2] Roennow B., Kielland-Brandt M.C. Yeast 9:1121-1130(1993).

[3] Brown L.J., McDonald M.J., Lehn D.A., Moran S.M. J. Biol. Chem. 269:14363-14366(1994).

794. NOL1/NOP2/sun family signature

The following proteins seems to be evolutionary related:

10 - Mammalian proliferating-cell nucleolar antigen p120 (gene NOL1) which may play a role in the regulation of the cell cycle and the increased nucleolar activity that is associated with the cell proliferation.

- Yeast nucleolar protein NOP2 (or YNA1) which could be involved in nucleolar function during the onset of growth, and in the maintenance of nucleolar structure.

- Yeast hypothetical protein YBL024w.

15 - Bacterial protein sun (also known as fmu).

- Escherichia coli hypothetical protein yebU.

- Mycobacterium tuberculosis hypothetical protein MtCY21B4.24.

- Methanococcus jannaschii hypothetical protein MJ0026.

20 NOL1 is a protein of 855 residues, NOP2 consists of 618 residues, YBL024w of 684, sun is a protein of about 430 to 450 residues and MJ026 has 274 residues. They share a conserved central domain which contains some highly conserved regions. One of these regions was selected as a signature pattern.

25 Consensus pattern[FV]-D-[KRA]-[LIVMA]-L-x-D-[AV]-P-C-[ST]-[GA]

795. moaA / nifB / pqqE family signature

A number of proteins involved in the biosynthesis of metallo cofactors have been shown [1,2] to be evolutionary related. These proteins are:

30 - Bacterial and archeobacterial protein moaA, which is involved in the biosynthesis of the molybdenum cofactor (molybdopterin; MPT).

- Arabidopsis thaliana cnx2, a protein involved in molybdopterin biosynthesis and which is highly similar to moaA.

- Bacillus subtilis narA, which seems to be the moaA ortholog in that bacteria.

- Bacterial protein nifB (or fixZ) which is involved in the biosynthesis of the nitrogenase iron-molybdenum cofactor.
- Bacterial protein pqqE which is involved in the biosynthesis of the cofactor pyrrolo-quinoline-quinone (PQQ).
- 5 - *Pyrococcus furiosus* cmo, a protein involved in the synthesis of a molybdopterin-based tungsten cofactor.
- *Caenorhabditis elegans* hypothetical protein F49E2.1.

10 All these proteins share, in their N-terminal region, a conserved domain that contains three cysteines. In moaA, these cysteines have been shown [1] to be important for the biological activity. They could be involved in the binding of an iron-sulfur cluster.

Consensus pattern[LIV]-x(3)-C-[NP]-[LIVMF]-[QRS]-C-x-[FYM]-C [The three C's are putative Fe-S ligands

- 15 [1] Menendez C., Igloi G., Henninger H., Brandsch R. Arch. Microbiol. 164:142-151(1995).
[2] Hoff T., Schnorr K.M., Meyer C., Caboche M. J. Biol. Chem. 270:6100-6107(1995).

796. Forkhead-associated (FHA) domain profile

20 The forkhead-associated (FHA) domain [1,E1] is a putative nuclear signalling domain found in a variety of otherwise unrelated proteins. The FHA domain comprise approximately 55 to 75 amino acids and contains three highly conserved blocks separated by divergent spacer regions. Currently it has been found in the following proteins:

- Four transcription factors that also contain a forkhead (FH) domain: mouse myocyte nuclear factor 1 (MNF1), yeast transcription factor FHL1, which probably controls pre-mRNA processing, and yeast FKH1 and FKH2. In those protein the FHA domain is located N-terminal of the DNA-binding FH domain.
- Kinase-associated protein phosphatase (KAPP) from *Arabidopsis thaliana*, a protein which specifically interacts with the receptor-type Ser/Thr-kinase RLK5. In KAPP, the FHA domain maps to a region that interacts with the receptor-type protein kinase RLK5 only if the kinase is phosphorylated on serine residues [2].
- 30 - Two protein kinases from yeast that are involved in mediating the nuclear response to DNA damage: DUN1 and SPK1/SAD1 [3]. The latter is the only known protein containing two copies of the FHA domain.

- Protein kinase cds1 from fission yeast contains a FHA domain and might be the ortholog of SPK1.

- Protein kinase MEK1 from yeast, which is involved in meiotic recombination.

- Human nuclear antigen Ki67 which is expressed only in proliferating cells.

5 - Yeast hypothetical protein YHR115c, which contains a RING-finger C-terminal of the FHA domain.

- Yeast hypothetical proteins L8083.1 and 9346.10, which contain an extensive coiled-coil region C-terminal of the FHA domain.

- Caenorhabditis elegans hypothetical protein ZK632.2.

10 - Caenorhabditis elegans hypothetical protein C01G6.5.

- FraH from the prokaryote Anabaena, which contains a zinc-finger motif N-terminal of the FHA domain.

- An ORF from the bacterium Streptomyces, which is on the opposite strand of the protein kinase pks1, overlapping the ORF of the kinase.

15 [1] Hofmann K.O., Bucher P. Trends Biochem. Sci. 20:347-349(1995).

[2] Stone J.M., Collinge M.A., Smith R.D., Horn M.A., Walker J.C. Science 266:793-795(1994).

[3] Navas T.A., Zhou Z., Elledge S.J. Cell 80:29-39(1995).

20 797. Ald_Xan_dh_C

Aldehyde oxidase and xanthine dehydrogenase, C terminus

[1] Romao MJ, Archer M, Moura I, Moura JJ, LeGall J, Engh R, Schneider M, Hof P, Huber R; Medline: 96072968 "Crystal structure of the xanthine oxidase-related aldehyde oxidoreductase from D. gigas." Science 1995;270:1170-1176.

Number of members: 54

30 798. Glyco_hydro_38

Glycosyl hydrolases family 38

Glycosyl hydrolases are key enzymes of carbohydrate metabolism.

Number of members: 20

[1] Henrissat B; Medline: 98313424; "Glycosidase families" Biochem Soc Trans 1998;26:153-156.

5 799. HECT

HECT-domain (ubiquitin-transferase).

The name HECT comes from Homologous to the E6-AP Carboxyl Terminus.

10 Number of members: 43

[1] Huibregtse JM, Scheffner M, Beaudenon S, Howley PM; Medline: 95223981; A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase." Proc Natl Acad Sci U S A 1995;92:2563-2567.

15

800. HRDC

HRDC domain

The HRDC (Helicase and RNase D C-terminal) domain has a putative role in nucleic acid binding. Mutations in the HRDC domain cause human disease.

20

Number of members: 19

[1] Morozov V, Mushegian AR, Koonin EV, Bork P; Medline: 98060076; A putative nucleic acid-binding domain in Bloom's and Werner's syndrome helicases" Trends Biochem Sci 1997;22:417-418.

25

801. Integrase

Integrase mediates integration of a DNA copy of the viral genome into the host chromosome. Integrase is composed of three domains. The amino-terminal domain is a zinc binding domain. The central domain is the catalytic domain [1].The carboxyl terminal domain is a DNA binding domain [2].

30

Number of members: 581

[1] Dyda F, Hickman AB, Jenkins TM, Engelman A, Craigie R, Davies DR; Medline: 95099322. Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases." Science 1994;266:1981-1986.

[2] Lodi PJ, Ernst JA, Kuszewski J, Hickman AB, Engelman A, Craigie R, Clore GM, Gronenborn AM; Medline: 95359147; Solution structure of the DNA binding domain of HIV-1 integrase." Biochemistry 1995;34:9826-9833

802. lig_chan

Ligand-gated ion channel

This family includes the four transmembrane regions of the ionotropic glutamate receptors and NMDA receptors.

Number of members: 128

[1] Tong G, Shepherd D, Jahr CE; Medline: 95184014; Synaptic desensitization of NMDA receptors by calcineurin." Science 1995;267:1510-1512.

803. RhoGAP

RhoGAP domain

GTPase activator proteins towards Rho/Rac/Cdc42-like small GTPases.

Number of members: 97

[1] Musacchio A, Cantley LC, Harrison SC; Medline: 97121392; Crystal structure of the breakpoint cluster region-homology domain from phosphoinositide 3-kinase p85 alpha subunit." Proc Natl Acad Sci U S A 1996;93:14373-14378.

[2] Barrett T, Xiao B, Dodson EJ, Dodson G, Ludbrook SB, Nurmahomed K, Gamblin SJ, Musacchio A, Smerdon SJ, Eccleston JF; Medline: 97162209; The structure of the GTPase-activating domain from p50rhoGAP." Nature 1997;385:458-461.

[3] Rittinger K, Walker PA, Eccleston JF, Nurmahomed K, Owen D, Laue E, Gamblin SJ, Smerdon SJ; Medline: 97404320; Crystal structure of a small G protein in complex with the GTPase-activating protein rhoGAP." Nature 1997;388:693-697.

[4] Boguski MS, McCormick F; Medline: 94081948; Proteins regulating Ras and its relatives." Nature 1993;366:643-654.

804. vwd

von Willebrand factor type D domain

- 5 [1] Bork P; Medline: 93327926; The modular architecture of a new family of growth regulators related to connective tissue growth factor." FEBS lett 1993;327:125-130.

Number of members: 92

10 805. zf-C4_Topoism

Topoisomerase DNA binding C4 zinc finger

- [1] Tse-Dinh YC, Beran-Steed RK; Medline: 89034032; Escherichia coli DNA topoisomerase I is a zinc metalloprotein with three repetitive zinc-binding domains." J Biol Chem 1988;263:15857-15859.
- 15 [2] Ahumada A, Tse-Dinh YC; Medline: 99011409; The Zn(II) binding motifs of E. coli DNA topoisomerase I is part of a high-affinity DNA binding domain." Biochem Biophys Res Commun 1998;251:509-514.

20 Number of members: 51

806. AIRC

AIR carboxylase

- 25 Members of this family catalyse the decarboxylation of 1-(5-phosphoribosyl)-5-amino-4-imidazole-carboxylate (AIR). This family catalyse the sixth step of de novo purine biosynthesis. Some members of this family contain two copies of this domain. Number of members: 35

30 807. Bromodomain signature and profile

PROSITE cross-reference(s): PS00633; BROMODOMAIN_1, PS50014;
BROMODOMAIN_2

The bromodomain [1,2,3] is a conserved region of about 70 amino acids found in the following proteins:

- Higher eukaryotes transcription initiation factor TFIID 250 Kd subunit (TBP-associated factor p250) (gene CCG1). P250 associated with the TFIID TATA-box binding protein and seems essential for progression of the G1 phase of the cell cycle.

- 5 - Human RING3, a protein of unknown function encoded in the MHC class II locus.
- Mammalian CREB-binding protein (CBP), which mediates cAMP-gene regulation by binding specifically to phosphorylated CREB protein.
- Drosophila female sterile homeotic protein (gene fsh), required maternally for proper expression of other homeotic genes involved in pattern formation, such as Ubx.
- 10 - Drosophila brahma protein (gene brm), a protein required for the activation of multiple homeotic genes.
- Mammalian homologs of brahma. In human, three brahma-like proteins are known: SNF2a(hBRM), SNF2b, and BRG1.
- Human BS69, a protein that binds to adenovirus E1A and inhibits E1A transactivation
- 15 - Human peregrin (or Br140).
- Yeast BDF1 [3], a transcription factor involved in the expression of a broad class of genes including snRNAs.
- Yeast GCN5, a general transcriptional activator operating in concert with certain other DNA-binding transcriptional activators, such as GCN4, HAP2/3/4 or ADA2.
- 20 - Yeast NPS1/STH1, involved in G(2) phase control in mitosis.
- Yeast SNF2/SWI2, which is part of a complex with the SNF5, SNF6, SWI3 and ADR6/SWI1 proteins. This SWI-complex is involved in transcriptional activation.
- Yeast SPT7, a transcriptional activator of Ty elements and possibly other genes.
- Caenorhabditis elegans protein cbp-1.
- 25 - Yeast hypothetical protein YGR056w.
- Yeast hypothetical protein YKR008w.
- Yeast hypothetical protein L9638.1.

Some proteins contain a region which, while similar to some extent to a classical
30 bromodomain, diverges from it by either lacking part of the domain or because of an insertion. These proteins are:

- Mammalian protein HRX (also known as All-1 or MLL), a protein involved in translocations leading to acute leukemias and which possibly acts as a transcriptional regulatory factor. HRX contains a region similar to the C- terminal half of the bromodomain.

- Caenorhabditis elegans hypothetical protein ZK783.4. The bromodomain of this protein has a 23 amino-acid insertion.

- Yeast protein YTA7. This protein contains a region with significant similarity to the C-terminal half of the bromodomain. As it is a member of the AAA family (see <PDOC00572>) it is also in a functionally different context.

The above proteins generally contain a single bromodomain, but some of them contain two copies, this is the case of BDF1, CCG1, fsh, RING3, YKR008w and L9638.1.

The exact function of this domain is not yet known but it is thought to be involved in protein-protein interactions and it may be important for the assembly or activity of multicomponent complexes involved in transcriptional activation.

The consensus pattern that has been developed spans a major part of the bromodomain; a more sensitive detection is available through the use of a profile which spans the whole domain.

Consensus pattern[STANVF]-x(2)-F-x(4)-[DNS]-x(5,7)-[DENQTF]-Y-[HFY]-x(2)-[LIVMFY]-x(3)-[LIVM]-x(4)-[LIVM]-x(6,8)-Y-x(12,13)-[LIVM]-x(2)-N-[SACF]-x(2)-[FY]

References

- [1] Haynes S.R., Doolard C., Winston F., Beck S., Trowsdale J., Dawid I.B. Nucleic Acids Res. 20:2693-2603(1992).
- [2] Tamkun J.W., Deuring R., Scott M.P., Kissinger M., Pattatucci A.M., Kaufman T.C., Kennison J.A. Cell 68:561-572(1992).
- [3] Tamkun J.W. Curr. Opin. Genet. Dev. 5:473-477(1995).

808. (CH) Actinin-type actin-binding domain signatures

PROSITE cross-reference(s): PS00019; ACTININ_1, PS00020; ACTININ_2

Alpha-actinin is a F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures [1]. The actin-binding domain of alpha-actinin seems to reside in the first 250 residues of the protein. A similar actin-binding domain has been found in the N-terminal region of many different actin-binding proteins [2,3]:

- In the beta chain of spectrin (or fodrin).
- In dystrophin, the protein defective in Duchenne muscular dystrophy (DMD) and which may play a role in anchoring the cytoskeleton to the plasma membrane.
- In the slime mold gelation factor (or ABP-120).
- In actin-binding protein ABP-280 (or filamin), a protein that link actin filaments to membrane glycoproteins.
- In fimbrin (or plastrin), an actin-bundling protein. Fimbrin differs from the above proteins in that it contains two tandem copies of the actin-binding domain and that these copies are located in the C-terminal part of the protein.

Two conserved regions were selected as signature patterns for this type of main. The first of this region is located at the beginning of the domain, while the second one is located in the central section and has been shown to be essential for the binding of actin.

Consensus pattern[EQ]-x(2)-[ATV]-[FY]-x(2)-W-x-N
 Consensus pattern[LIVM]-x-[SGN]-[LIVM]-[DAGHE]-[SAG]-x-[DNEAG]-[LIVM]-x-[DEAG]-x(4)-[LIVM]-x-[LM]-[SAG]-[LIVM]-[LIVMT]-W-x-[LIVM](2)

[1] Schleicher M., Andre E., Harmann A., Noegel A.A. Dev. Genet. 9:521-530(1988).

[2] Matsudaira P. Trends Biochem. Sci. 16:87-92(1991).

[3] Dubreuil R.R. BioEssays 13:219-226(1991).

809. (COX1) Heme-copper oxidase subunit I, copper B binding region signature
 PROSITE cross-reference(s): PS00077; COX1

Heme-copper respiratory oxidases [1] are oligomeric integral membrane protein complexes that catalyze the terminal step in the respiratory chain: they transfer electrons from cytochrome c or a quinol to oxygen. Some terminal oxidases generate a transmembrane proton gradient across the plasma membrane (prokaryotes) or the mitochondrial inner membrane (eukaryotes). The enzyme

complex consists of 3-4 subunits (prokaryotes) up to 13 polypeptides (mammals) of which only the catalytic subunit (equivalent to mammalian subunit 1 (CO I)) is found in all heme-copper respiratory oxidases. The presence of a bimetallic center (formed by a high-spin heme and copper B) as well as a low-spin heme, both ligated to six conserved histidine residues near the outer side of four transmembrane spans within CO I is common to all family members [2-4].

In contrary to eukaryotes the respiratory chain of prokaryotes is branched to multiple terminal oxidases. The enzyme complexes vary in heme and copper composition, substrate type and substrate affinity. The different respiratory oxidases allow the cells to customize their respiratory systems according a variety of environmental growth conditions [1].

Recently also a component of an anaerobic respiratory chain has been found to contain the copper B binding signature of this family: nitric oxide reductase (NOR) exists in denitrifying species of Archae and Eubacteria.

Enzymes that belong to this family are:

- Mitochondrial-type cytochrome c oxidase (EC 1.9.3.1) which uses cytochrome c as electron donor. The electrons are transferred via copper A (Cu(A)) and heme a to the bimetallic center of CO I that is formed by a penta-coordinated heme a and copper B (Cu(B)). Subunit 1 contains 12 transmembrane regions. Cu(B) is said to be ligated to three of the conserved histidine residues within the transmembrane segments 6 and 7.
- Quinol oxidase from prokaryotes that transfers electrons from a quinol to the binuclear center of polypeptide I. This category of enzymes includes Escherichia coli cytochrome O terminal oxidase complex which is a component of the aerobic respiratory chain that predominates when cells are grown at high aeration.
- FixN, the catalytic subunit of a cytochrome c oxidase expressed in nitrogen-fixing bacteroids living in root nodules. The high affinity for oxygen allows oxidative phosphorylation under low oxygen concentrations. A similar enzyme has been found in other purple bacteria.

- Nitric oxide reductase (EC 1.7.99.7) from *Pseudomonas stutzeri*. NOR reduces nitrate to dinitrogen. It is a heterodimer of norC and the catalytic subunit norB. The latter contains the 6 invariant histidine residues and 12 transmembrane segments [5].

5

As a signature pattern the copper-binding region was used.

Consensus pattern[YWG]-[LIVFYWTA](2)-[VGS]-H-[LNP]-x-V-x(44,47)-H-H [The three H's are copper B ligands]

10

Notcytochrome bd complexes do not belong to this family.

[1]

Garcia-Horsman J.A., Barquera B., Rumbley J., Ma J., Gennis R.B.
J. Bacteriol. 176:5587-5600(1994).

15

[2]

Castresana J., Luebben M., Saraste M., Higgins D.G.
EMBO J. 13:2516-2525(1994).

[3]

Capaldi R.A., Malatesta F., Darley-USmar V.M.
Biochim. Biophys. Acta 726:135-148(1983).

20

[4]

Holm L., Saraste M., Wikstrom M.
EMBO J. 6:2819-2823(1987).

25

[5]

Saraste M., Castresana J.
FEBS Lett. 341:1-4(1994).

810. (dehydrog_molyb) Eukaryotic molybdopterin oxidoreductases signature
PROSITE cross-reference(s): PS00559; MOLYBDOPTERIN_EUK

30

A number of different eukaryotic oxidoreductases that require and bind a molybdopterin cofactor have been shown [1] to share a few regions of sequence similarity. These enzymes are:

- Xanthine dehydrogenase (EC 1.1.1.204), which catalyzes the oxidation of xanthine to uric acid with the concomitant reduction of NAD. Structurally, this enzyme of about 1300 amino acids consists of at least three distinct domains: an N-terminal 2Fe-2S ferredoxin-like iron-sulfur binding domain (see <PDOC00175>), a central FAD/NAD-binding domain and a C-terminal Mo-pterin domain.

- Aldehyde oxidase (EC 1.2.3.1), which catalyzes the oxidation aldehydes into acids. Aldehyde oxidase is highly similar to xanthine dehydrogenase in its sequence and domain structure.

- Nitrate reductase (EC 1.6.6.1), which catalyzes the reduction of nitrate to nitrite. Structurally, this enzyme of about 900 amino acids consists of an N-terminal Mo-pterin domain, a central cytochrome b5-type heme-binding domain (see <PDOC00170>) and a C-terminal FAD/NAD-binding cytochrome reductase domain.

- Sulfite oxidase (EC 1.8.3.1), which catalyzes the oxidation of sulfite to sulfate. Structurally, this enzyme of about 460 amino acids consists of an N-terminal cytochrome b5-binding domain followed by a Mo-pterin domain.

There are a few conserved regions in the sequence of the molybdopterin-binding domain of these enzymes. The pattern uses to detect these proteins is based on one of them. It contains a cysteine residue which could be involved in binding the molybdopterin cofactor.

Consensus pattern[GA]-x(3)-[KRNOHT]-x(11,14)-[LIVMFYWS]-x(8)-[LIVMF]-x-C-x(2)-[DEN]-R-x(2)-[DE]

[1]

Wootton J.C., Nicolson R.E., Cock J.M., Walters D.E., Burke J.F., Doyle W.A., Bray R.C.
Biochim. Biophys. Acta 1057:157-185(1991).

811. (DNA_ligase) ATP-dependent DNA ligase signatures

PROSITE cross-reference(s): PS00697; DNA_LIGASE_A1, PS00333; DNA_LIGASE_A2

DNA ligase (polydeoxyribonucleotide synthase) is the enzyme that joins two DNA fragments by catalyzing the formation of an internucleotide ester bond between phosphate and deoxyribose. It is active during DNA replication, DNA repair and

- 5 DNA recombination. There are two forms of DNA ligase: one requires ATP (EC 6.5.1.1), the other NAD (EC 6.5.1.2).

Eukaryotic, archaebacterial, virus and phage DNA ligases are ATP-dependent. During the first step of the joining reaction, the ligase interacts with ATP
10 to form a covalent enzyme-adenylate intermediate. A conserved lysine residue is the site of adenylation [1,2].

Apart from the active site region, the only conserved region common to all ATP-dependent DNA ligases is found [3] in the C-terminal section and contains
15 a conserved glutamate as well as four positions with conserved basic residues.

Signature patterns were developed for both conserved regions.

Consensus pattern[EDQH]-x-K-x-[DN]-G-x-R-[GACIVM] [K is the active site
20 residue]

Consensus patternE-G-[LIVMA]-[LIVM](2)-[KR]-x(5,8)-[YW]-[ONEK]-x(2,6)-
[KRH]-x(3,5)-K-[LIVMFY]-K

- 25 Sequences known to belong to this class detected by the patternALL, except for archaebacterial DNA ligases.

[1]

Tomkinson A.E., Totty N.F., Ginsburg M., Lindahl T.
Proc. Natl. Acad. Sci. U.S.A. 88:400-404(1991).

- 30 [2]

Lindahl T., Barnes D.E.

Annu. Rev. Biochem. 61:251-281(1992).

[3]

Kletzin A.

Nucleic Acids Res. 20:5389-5396(1992).

812. (FAD_Gly3P_dh) FAD-dependent glycerol-3-phosphate dehydrogenase signatures
PROSITE cross-reference(s): PS00977; FAD_G3PDH_1, PS00978; FAD_G3PDH_2

5

FAD-dependent glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) (GPD) catalyzes the conversion of glycerol-3-phosphate into dihydroxyacetone phosphate. In bacteria [1] it is associated with the utilization of glycerol coupled to respiration. In *Escherichia coli*, two isozymes are known: one expressed under anaerobic conditions (gene *glpA*) and one in aerobic conditions (gene *glpD*). In eukaryotes, a mitochondrial form of GPD participates in the glycerol phosphate shuttle in conjunction with an NAD-dependent cytoplasmic GPD (EC 1.1.1.8) [2, 3].

10

15

These enzymes are proteins of about 60 to 70 Kd which contain a probable FAD-binding domain in their N-terminal extremity. The mammalian enzyme differs from the bacterial or yeast proteins by having an EF-hand calcium-binding region (See <PDOC00018>) in its C-terminal extremity.

20

Two signature patterns were developed. One based on the first half of the FAD-binding domain and one which corresponds to a conserved region in the central part of these enzymes.

Consensus pattern[IV]-G-G-G-x(2)-G-[STACV]-G-x-A-x-D-x(3)-R-G

25

Consensus patternG-G-K-x(2)-[GSTE]-Y-R-x(2)-A

[1]

Austin D., Larson T.J.

J. Bacteriol. 173:101-107(1991).

30

[2]

Roennow B., Kielland-Brandt M.C.

Yeast 9:1121-1130(1993).

[3]

Brown L.J., McDonald M.J., Lehn D.A., Moran S.M.

J. Biol. Chem. 269:14363-14366(1994).

813. (Fapy_DNA_glyco) Formamidopyrimidine-DNA glycosylase signature

PROSITE cross-reference(s): PS01242; FPG

5

Formamidopyrimidine-DNA glycosylase (EC 3.2.2.23) [1] (Fapy-DNA glycosylase)

(gene *fpg*) is a bacterial enzyme involved in DNA repair and which excise oxidized purine bases to release 2,6-diamino-4-hydroxy-5N-methylformamido-pyrimidine (Fapy) and 7,8-dihydro-8-oxoguanine (8-OxoG) residues. In addition
10 to its glycosylase activity, FPG can also nick DNA at apurinic/apyrimidinic sites (AP sites). FPG is a monomeric protein of about 32 Kd which binds and require zinc for its activity.

The binding site for zinc seems to be located in the C-terminal part of the

15 enzyme where four conserved and essential [2] cysteines are located. A signature pattern was developed based on this region.

Consensus pattern C-x(2,4)-C-x-[GTAQ]-x-[IV]-x(7)-R-[GSTAN]-[STA]-x-[FYI]-C-x(2)-C-Q

20 [The four C's are putative zinc ligands]

[1]

Duwat P., de Oliveira R., Ehrlich S.D., Boiteux S.
Microbiology 141:411-417(1995).

25 [2]

O'Connor T.E., Graves R.J., Demurcia G., Castaing B., Laval J.
J. Biol. Chem. 268:9063-9070(1993).

814. (G_glu_transpept) Gamma-glutamyltranspeptidase signature

30 PROSITE cross-reference(s): PS00462; G_GLU_TRANSPEPTIDASE

Gamma-glutamyltranspeptidase (EC 2.3.2.2) (GGT) [1] catalyzes the transfer of the gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate). GGT plays a key role in the

gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione. In prokaryotes and eukaryotes, it is an enzyme that consists of two polypeptide chains, a heavy and a light subunit, processed from a single chain precursor. The active site of GGT is known to be located in the light subunit.

The sequences of mammalian and bacterial GGT show a number of regions of high similarity [2]. *Pseudomonas cephalosporin acylases* (EC 3.5.1.-) that convert 7-beta-(4-carboxybutanamido)-cephalosporanic acid (GL-7ACA) into 7-aminocephalosporanic acid (7ACA) and glutaric acid are evolutionary related to GGT and also show some GGT activity [3]. Like GGT, these GL-7ACA acylases, are also composed of two subunits.

One of the conserved regions correspond to the N-terminal extremity of the mature light chains of these enzymes. This region was used as a signature pattern.

Consensus pattern T-[STA]-H-x-[ST]-[LIVMA]-x(4)-G-[SN]-x-V-[STA]-x-T-x-T-[LIVM]-[NE]-x(1,2)-[FY]-G

[1]

Tate S.S., Meister A.
Meth. Enzymol. 113:400-419(1985).

[2]

Suzuki H., Kumagai H., Echigo T., Tochikura T.
J. Bacteriol. 171:5169-5172(1989).

[3]

Ishiye M., Niwa M.
Biochim. Biophys. Acta 1132:233-239(1992).

815. G-protein gamma subunit profile

PROSITE cross-reference(s): PS50058; G_PROTEIN_GAMMA

Guanine nucleotide-binding proteins (G proteins) [1] act as intermediaries in

the transduction of signals generated by transmembrane receptors. G proteins consist of three subunits (alpha, beta, and gamma). The alpha subunit binds to and hydrolyzes GTP; the functions of the beta and gamma subunits are less clear but they seem to be required for the replacement of GDP by GTP as well as for membrane anchoring and receptor recognition.

The gamma subunits are small proteins (from 70 to 110 residues) that are bound to the membrane via a isoprenyl group (either a farnesyl or a geranyl-geranyl) covalently linked to their C-terminus. In mammals there are at least 12 different isoforms of gamma subunits.

The *Caenorhabditis elegans* protein egl-10, which is a regulator of G-protein signalling, contains a G-protein gamma-like domain.

A profile was developed that spans the complete length of the gamma subunit.

[1]

Pennington S.R.

Protein Prof. 2:16-315(1995).

816. GNS1/SUR4 family signature

PROSITE cross-reference(s): PS01188; GNS1_SUR4

The following group of eukaryotic integral membrane proteins, whose exact function has not yet clearly been established, are evolutionary related [1]:

- Yeast GNS1 [2], a protein involved in synthesis of 1,3-beta-glucan.
- Yeast SUR4 (or APA1, SRE1) [3], a protein that could act in a glucose-signaling pathway that controls the expression of several genes that are transcriptionally regulated by glucose.
- Yeast hypothetical protein YJL196c.
- *Caenorhabditis elegans* hypothetical protein C40H1.4.
- *Caenorhabditis elegans* hypothetical protein D2024.3.

The proteins have from 290 to 435 amino acid residues. Structurally, they seem to be formed of three sections: a N-terminal region with two transmembrane domains, a central hydrophilic loop and a C-terminal region that contains from one to three transmembrane domains. A conserved region that contains three histidines was selected as a signature pattern. This region is located in the hydrophilic loop.

Consensus pattern L-x-F-L-H-x-Y-H-H

[1]

Bairoch A.

Unpublished observations (1996).

[2]

El-Sherbeini M., Clemas J.A.

J. Bacteriol. 177:3227-3234(1995).

[3]

Garcia-Arranz M., Maldonado A.M., Mazon M.J., Portillo F.

J. Biol. Chem. 269:18076-18082(1994).

817. Immunoglobulins and major histocompatibility complex proteins signature
PROSITE cross-reference(s): PS00290; IG_MHC

The basic structure of immunoglobulin (Ig) [1] molecules is a tetramer of two light chains and two heavy chains linked by disulfide bonds. There are two types of light chains: kappa and lambda, each composed of a constant domain (CL) and a variable domain (VL). There are five types of heavy chains: alpha, delta, epsilon, gamma and mu, all consisting of a variable domain (VH) and three (in alpha, delta and gamma) or four (in epsilon and mu) constant domains (CH1 to CH4).

The major histocompatibility complex (MHC) molecules are made of two chains. In class I [2] the alpha chain is composed of three extracellular domains, a transmembrane region and a cytoplasmic tail. The beta chain (beta-2-

microglobulin) is composed of a single extracellular domain. In class II [3], both the alpha and the beta chains are composed of two extracellular domains, a transmembrane region and a cytoplasmic tail.

- 5 It is known [4,5] that the Ig constant chain domains and a single extracellular domain in each type of MHC chains are related. These homologous domains are approximately one hundred amino acids long and include a conserved intradomain disulfide bond. A small pattern around the C-terminal cysteine is involved in this disulfide bond which can be used to detect
10 these category of Ig related proteins.

- Consensus pattern[FY]-x-C-x-[VA]-x-H-Sequences known to belong to this class detected by the pattern: Ig heavy chains type Alpha C region : All, in CH2 and CH3. Ig heavy chains type Delta C region : All, in CH3. Ig
15 heavy chains type Epsilon C region: All, in CH1, CH3 and CH4. Ig heavy chains type Gamma C region : All, in CH3 and also CH1 in some cases Ig heavy chains type Mu C region : All, in CH2, CH3 and CH4. Ig light chains type Kappa C region : In all CL except rabbit and Xenopus. Ig light chains type Lambda C region : In all CL except rabbit. MHC class I alpha chains :
20 All, in alpha-3 domains, including in the cytomegalovirus MHC-I homologous protein [6]. Beta-2-microglobulin : All. MHC class II alpha chains: All, in alpha-2 domains. MHC class II beta chains: All, in beta-2 domains.

[1]

- 25 Gough N.
Trends Biochem. Sci. 6:203-205(1981).

[2]

Klein J., Figueroa F.
Immunol. Today 7:41-44(1986).

- 30 [3]

Figueroa F., Klein J.
Immunol. Today 7:78-81(1986).

[4]

Orr H.T., Lancet D., Robb R.J., Lopez de Castro J.A., Strominger J.L.

Nature 282:266-270(1979).

[5]

Cushley W., Owen M.J.

Immunol. Today 4:88-92(1983).

5 [6]

Beck S., Barrel B.G.

Nature 331:269-272(1988).

318. (IGFBP) Insulin-like growth factor binding proteins signature

10 PROSITE cross-reference(s): PS00222; IGF_BINDING

The insulin-like growth factors (IGF-I and IGF-II) bind to specific binding proteins in extracellular fluids with high affinity [1,2,3]. These IGF-binding proteins (IGFBP) prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cells culture. They seem to alter the interaction of IGFs with their cell surface receptors. There are at least six different IGFBPs and they are structurally related.

20 The following growth-factor inducible proteins are structurally related to IGFBPs and could function as growth-factor binding proteins [4,5]:

- Mouse protein cyr61 and its probable chicken homolog, protein CEF-10.
- Human connective tissue growth factor (CTGF) and its mouse homolog, protein FISP-12.
- Vertebrate protein NOV.

As a signature pattern a conserved cysteine-rich region located in the N-terminal section of these proteins is used.

Consensus pattern G-C-[GS]-C-C-x(2)-C-A-x(6)-C

Sequences known to belong to this class detected by the pattern ALL, except for IGFBP-6's.

[1]

Rechler M.M.

Vitam. Horm. 47:1-114(1993).

[2]

5 Shimasaki S., Ling N.

Prog. Growth Factor Res. 3:243-266(1991).

[3]

Clemmons D.R.

Trends Endocrinol. Metab. 1:412-417(1990).

10 [4]

Bradham D.M., Igarashi A., Potter R.L., Grotendorst G.R.

J. Cell Biol. 114:1285-1294(1991).

[5]

Maloisel V., Martinerie C., Dambrine G., Plassiat G., Brisac M., Crochet

15 J., Perbal B.

Mol. Cell. Biol. 12:10-21(1992).

819. LMWpC : Low molecular weight phosphotyrosine protein phosphatase

Number of members: 34

20

[1]Medline: 94329182, The crystal structure of a low-molecular-weight phosphotyrosine protein phosphatase. Su XD, Taddei N, Stefani M, Ramponi G, Nordlund P; Nature 1994;370:575-578.

25 820. (myosin_head) ATP/GTP-binding site motif A (P-loop)

PROSITE cross-reference(s): PS00017; ATP_GTP_A

From sequence comparisons and crystallographic data analysis it has been shown [1,2,3,4,5,6] that an appreciable proportion of proteins that bind ATP or GTP share a number of more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region, which typically forms a flexible loop between a beta-strand and an alpha-helix. This loop interacts with one of the phosphate groups of the nucleotide. This sequence motif is generally referred to as the 'A' consensus sequence [1] or the 'P-loop' [5].

30

There are numerous ATP- or GTP-binding proteins in which the P-loop is found. A number of protein families for which the relevance of the presence of such motif has been noted is listed below:

5

- ATP synthase alpha and beta subunits (see <PDOC00137>).
- Myosin heavy chains.
- Kinesin heavy chains and kinesin-like proteins (see <PDOC00343>).
- Dynamins and dynamin-like proteins (see <PDOC00362>).
- 10 - Guanylate kinase (see <PDOC00670>).
- Thymidine kinase (see <PDOC00524>).
- Thymidylate kinase (see <PDOC01034>).
- Shikimate kinase (see <PDOC00868>).
- Nitrogenase iron protein family (nifH/frxC) (see <PDOC00580>).
- 15 - ATP-binding proteins involved in 'active transport' (ABC transporters) [7] (see <PDOC00185>).
- DNA and RNA helicases [8,9,10].
- GTP-binding elongation factors (EF-Tu, EF-1alpha, EF-G, EF-2, etc.).
- Ras family of GTP-binding proteins (Ras, Rho, Rab, Ral, Ypt1, SEC4, etc.).
- 20 - Nuclear protein ran (see <PDOC00859>).
- ADP-ribosylation factors family (see <PDOC00781>).
- Bacterial dnaA protein (see <PDOC00771>).
- Bacterial recA protein (see <PDOC00131>).
- Bacterial recF protein (see <PDOC00539>).
- 25 - Guanine nucleotide-binding proteins alpha subunits (Gi, Gs, Gt, G0, etc.).
- DNA mismatch repair proteins mutS family (See <PDOC00388>).
- Bacterial type II secretion system protein E (see <PDOC00567>).

30

Not all ATP- or GTP-binding proteins are picked-up by this motif. A number of proteins escape detection because the structure of their ATP-binding site is completely different from that of the P-loop. Examples of such proteins are the E1-E2 ATPases or the glycolytic kinases. In other ATP- or GTP-binding proteins the flexible loop exists in a slightly different form; this is the case for tubulins or protein kinases. A special mention must be reserved for

adenylate kinase, in which there is a single deviation from the P-loop pattern: in the last position Gly is found instead of Ser or Thr.

Consensus pattern[AG]-x(4)-G-K-[ST]

5

[1]

Walker J.E., Saraste M., Runswick M.J., Gay N.J.
EMBO J. 1:945-951(1982).

[2]

10 Moller W., Amons R.
FEBS Lett. 186:1-7(1985).

[3]

Fry D.C., Kuby S.A., Mildvan A.S.
Proc. Natl. Acad. Sci. U.S.A. 83:907-911(1986).

15

[4]

Dever T.E., Glynias M.J., Merrick W.C.
Proc. Natl. Acad. Sci. U.S.A. 84:1814-1818(1987).

[5]

20 Saraste M., Sibbald P.R., Wittinghofer A.
Trends Biochem. Sci. 15:430-434(1990).

[6]

Koonin E.V.
J. Mol. Biol. 229:1165-1174(1993).

[7]

25 Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P.
J. Bioenerg. Biomembr. 22:571-592(1990).

[8]

30 Hodgman T.C.
Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).

[9]

Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P.
Nature 337:121-122(1989).

[10]

Gorbalenya A.E., Koonin E.V., Donchenko A.P., Blinov V.M.

Nucleic Acids Res. 17:4713-4730(1989).

5 821. PE: PE family

This family named after a PE motif near to the amino terminus of the domain. The PE family of proteins all contain an amino-terminal region of about 110 amino acids. The carboxyl terminus of this family are variable and fall into several classes. The largest class of PE proteins is the highly repetitive PGRS class which have a high glycine content. The function of these proteins is uncertain but it has been suggested that they may be related to antigenic variation of *Mycobacterium tuberculosis* [1]. Number of members: 88

[1] Medline: 98295987. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Barrell BG, et al; Nature 1998;393:537-544.

822. (RNB) Ribonuclease II family signature

20 PROSITE cross-reference(s): PS01175; RIBONUCLEASE_II

On the basis of sequence similarities, the following bacterial and eukaryotic proteins seem to form a family:

- 25 - *Escherichia coli* and related bacteria ribonuclease II (EC 3.1.13.1) (RNase II) (gene *rnB*) [1]. RNase II is an exonuclease involved in mRNA decay. It degrades mRNA by hydrolyzing single-stranded polyribonucleotides processively in the 3' to 5' direction.
- Bacterial protein *vacB*. In *Shigella flexneri*, *vacB* has been shown to be required for the expression of virulence genes at the posttranscriptional level.
- Yeast protein SSD1 (or SRK1) which is implicated in the control of the cell cycle G1 phase.
- Yeast protein DIS3 [2], which binds to ran (GSP1) and enhances the

nucleotide-releasing activity of RCC1 on ran.

- Fission yeast protein dis3, which is implicated in mitotic control.

- Neurospora crassa cyt-4, a mitochondrial protein required for RNA 5' and 3' end processing and splicing.

5 - Yeast protein MSU1, which is involved in mitochondrial biogenesis.

- Synechocystis strain PCC 6803 protein zam [3], which control resistance to the carbonic anhydrase inhibitor acetazolamide.

- Caenorhabditis elegans hypothetical protein F48E8.6.

10 The size of these proteins range from 644 residues (rnb) to 1250 (SSD1). While their sequence is highly divergent they share a conserved domain in their C-terminal section [4]. It is possible that this domain plays a role in a putative exonuclease function that would be common to all these proteins. A signature pattern was developed based on the core of this conserved domain.

15 Consensus pattern[HI]-[FYE]-[GSTAM]-[LIVM]-x(4,5)-Y-[STAL]-x-[FWVAC]-[TV]-[SA]-P-[LIVMA]-[RQ]-[KR]-[FY]-x-D-x(3)-[HQ]

[1]

20 Zilhao R., Camelo L., Arraiano C.M.
Mol. Microbiol. 8:43-51(1993).

[2]

Noguchi E., Hayashi N., Azuma Y., Seki T., Nakamura M., Nakashima N., Yanagida M., He X., Mueller U., Sazer S., Nishimoto T.

25 EMBO J. 15:5595-5605(1996).

[3]

Beuf L., Bedu S., Cami B., Joset F.
Plant Mol. Biol. 27:779-788(1995).

[4]

30 Mian I.S.
Nucleic Acids Res. 25:3187-3195(1997).

823. Src homology 2 (SH2) domain profile

PROSITE cross-reference(s): PS50001; SH2

The Src homology 2 (SH2) domain is a protein domain of about 100 amino-acid residues first identified as a conserved sequence region between the oncoproteins Src and Fps [1]. Similar sequences were later found in many other intracellular signal-transducing proteins [2]. SH2 domains function as regulatory modules of intracellular signalling cascades by interacting with high affinity to phosphotyrosine-containing target peptides in a sequence-specific and strictly phosphorylation-dependent manner [3,4,5,6].

The SH2 domain has a conserved 3D structure consisting of two alpha helices and six to seven beta-strands. The core of the domain is formed by a continuous beta-meander composed of two connected beta-sheets [7].

So far, SH2 domains have been identified in the following proteins:

- Many vertebrate, invertebrate and retroviral cytoplasmic (non-receptor) protein tyrosine kinases. In particular in the Src, Abl, Btk, Csk and ZAP70 families of kinases.
- Mammalian phosphatidylinositol-specific phospholipase C gamma-1 and -2. Two copies of the SH2 domain are found in those proteins in between the catalytic 'X-' and 'Y-boxes' (see <PDOC50007>).
- Mammalian phosphatidyl inositol 3-kinase regulatory p85 subunit.
- Some vertebrate and invertebrate protein-tyrosine phosphatases.
- Mammalian Ras GTPase-activating protein (GAP).
- Adaptor proteins mediating binding of guanine nucleotide exchange factors to growth factor receptors: vertebrate GRB2, Caenorhabditis elegans sem-5 and Drosophila DRK.
- Mammalian Vav oncoprotein, a guanine-nucleotide exchange factor of the CDC24 family.
- Miscellaneous proteins interacting with vertebrate receptor protein tyrosine kinases: oncoprotein Crk, mammalian cytoplasmic proteins Nck, Shc.
- STAT proteins (signal transducers and activators of transcription).
- Chicken tensin.
- Yeast transcriptional control protein SPT6.

The profile developed to detect SH2 domains is based on a structural alignment consisting of 8 gap-free blocks and 7 linker regions totaling 92 match positions.

5

[1]

Sadowski I., Stone J.C., Pawson T.
Mol. Cell. Biol. 6:4396-4408(1986).

[2]

10 Russel R.B., Breed J., Barton G.J.
FEBS Lett. 304:15-20(1992).

[3]

Marangere L.E.M., Pawson T.
J. Cell Sci. Suppl. 18:97-104(1994).

15

[4]

Pawson T., Schlessinger J.
Curr. Biol. 3:434-442(1993).

[5]

Mayer B.J., Baltimore D.
20 Trends Cell. Biol. 3:8-13(1993).

[6]

Pawson T.
Nature 373:573-580(1995).

[7]

25 Kuriyan J., Cowburn D.
Curr. Opin. Struct. Biol. 3:828-837(1993).

824. Sulfate transporters signature

PROSITE cross-reference(s): PS01130; SULFATE_TRANSF

30

A number of proteins involved in the transport of sulfate across a membrane as well as some yet uncharacterized proteins have been shown [1,2] to be evolutionary related. These proteins are:

- *Neurospora crassa* sulfate permease II (gene *cys-14*).
- Yeast sulfate permeases (genes *SUL1* and *SUL2*).
- Rat sulfate anion transporter 1 (*SAT-1*).
- Mammalian *DTDST*, a probable sulfate transporter which, in Human, is
- 5 involved in the genetic disease, diastrophic dysplasia (*DTD*).
- Sulfate transporters 1, 2 and 3 from the legume *Stylosanthes hamata*.
- Human pendrin (gene *PDS*), which is involved in a number of hearing loss genetic diseases.
- 10 - Human protein *DRA* (Down-Regulated in Adenoma).
- Soybean early nodulin 70.
- *Escherichia coli* hypothetical protein *ychM*.
- *Caenorhabditis elegans* hypothetical protein *F41D9.5*.
- 15 As expected by their transport function, these proteins are highly hydrophobic and seem to contain about 12 transmembrane domains. The best conserved region seems to be located in the second transmembrane region and is used as a signature pattern.
- 20 Consensus pattern [PAV]-x-Y-[GS]-L-Y-[STAG](2)-x(4)-[LIVFYA]-[LIVST]-[YI]-x(3)-[GA]-[GST]-S-[KR]
- [1]
Sandal N.N., Marcker K.A.
- 25 Trends Biochem. Sci. 19:19-19(1994).
- [2]
Smith F.W., Hawkesford M.J., Prosser I.M., Clarkson D.T.
- Mol. Gen. Genet. 247:709-715(1995).
- 30 825. TYA: TYA transposon protein
Ty are yeast transposons. A 5.7kb transcript codes for p3 a fusion protein of TYA and TYB. The TYA protein is analogous to the gag protein of retroviruses. TYA a is cleaved to form 46kd protein which can form mature virion like particles [1]. Number of members: 59

[1] Medline: 97404699. Cryo-electron microscopy structure of yeast Ty retrotransposon virus-like particles. Palmer KJ, Tichelaar W, Myers N, Burns NR, Butcher SJ, Kingsman AJ, Fuller SD, Saibil HR; J Virol 1997;71:6863-6868.

5 826. Aldolase_II

Class II Aldolase and Adducin N-terminal domain.

-!- This family includes class II aldolases and adducins which have not been ascribed any enzymatic function. Number of members: 37

10 References:

[1] Medline: 93294819. The spatial structure of the class II L-fucose-1-phosphate aldolase from *Escherichia coli*. Dreyer MK, Schulz GE; J Mol Biol 1993;231:549-553.

[2] Medline: 96256522. Catalytic mechanism of the metal-dependent fucose aldolase from *Escherichia coli* as derived from the structure. Dreyer MK, Schulz GE; J Mol Biol 1996;259:458-466.

827. CBD_2

-!- Two tryptophan residues are involved in cellulose binding.

-!- Cellulose binding domain found in bacteria. Number of members: 51

20 References:

[1] Medline: 95284032. Solution structure of a cellulose-binding domain from *Cellulomonas fimi* by nuclear magnetic resonance spectroscopy. Xu GY, Ong E, Gilkes NR, Kilburn DG, Muhandiram DR, Harris-Brandts M, Carver JP, Kay LE, Harvey TS; Biochemistry 1995;34:6993-7009.

828. P

A unique feature of the eukaryotic subtilisin-like proprotein convertases is the presence of an additional highly conserved sequence of approximately 150 residues (P domain) located immediately downstream of the catalytic domain.

Number of members: 91

References:

[1] Medline: 94252314. A C-terminal domain conserved in precursor processing proteases is required for intramolecular N-terminal maturation of pro-Kex2 protease. Gluschkof P, Fuller RS; EMBO J 1994;13:2280-2288.

[2] Medline: 98225190. Regulatory roles of the P domain of the subtilisin-like prohormone convertases. Zhou A, Martin S, Lipkind G, LaMendola J, Steiner DF; J Biol Chem 1998;273:11107-11114.

829. Uncharacterized protein family UPF0020 signature

PROSITE cross-reference(s): PS01261; UPF0020

The following uncharacterized proteins have been shown [1] to share regions of similarities:

- Escherichia coli hypothetical protein ycbY and HI0116/15, the corresponding Haemophilus influenzae protein.

- Bacillus subtilis hypothetical protein ypsC.

- Synechocystis strain PCC 6803 hypothetical protein slr0064.

- Methanococcus jannaschii hypothetical proteins MJ0438 and MJ0710.

These are hydrophilic proteins of from 40 Kd to about 80 Kd. They can be picked up in the database by the following pattern.

Consensus patternD-P-[LIVMF]-C-G-[ST]-G-x(3)-[LI]-E

References:

[1] Bairoch A. Unpublished observations (1997).

830. Uncharacterized protein family UPF0031 signatures

PROSITE cross-reference(s): PS01049; UPF0031_1; PS01050; UPF0031_2

The following uncharacterized proteins have been shown [1] to share regions of similarities:

- Yeast chromosome XI hypothetical protein YKL151c.

- Caenorhabditis elegans hypothetical protein R107.2.

- Escherichia coli hypothetical protein yjeF.

- *Bacillus subtilis* hypothetical protein yxkO.
- *Helicobacter pylori* hypothetical protein HP1363.
- *Mycobacterium tuberculosis* hypothetical protein MtCY77.05c.
- *Mycobacterium leprae* hypothetical protein B229_C2_201.
- 5 - *Synechocystis* strain PCC 6803 hypothetical protein sl1433.
- *Methanococcus jannaschii* hypothetical protein MJ1586.

These are proteins of about 30 to 40 Kd whose central region is well conserved. They can be picked up in the database by the following patterns.

10

Consensus pattern[SAV]-[IVW]-[LVA]-[LIV]-G-[PNS]-G-L-[GP]-x-[DENQT]

Consensus pattern[GA]-G-x-G-D-[TV]-[LT]-[STA]-G-x-[LIVM]

831. (ACOX)

15 Acyl-CoA oxidase

This is a family of Acyl-CoA oxidases EC:1.3.3.6. Acyl-coA oxidase converts acyl-CoA into trans-2-enoyl-CoA [1].

20 Number of members: 39

[1] Hayashi H, De Bellis L, Yamaguchi K, Kato A, Hayashi M, Nishimura M; Medline: 98192624. Molecular characterization of a glyoxysomal long chain acyl-CoA oxidase that is synthesized as a precursor of higher molecular mass in pumpkin." J Biol Chem 1998;273:8301-8307.

25

832. (AICARFT_IMPCHas)

AICARFT/IMPCHase bienzyme

30

This is a family of bifunctional enzymes catalysing the last steps in de novo purine biosynthesis. The bifunctional enzyme is found in both prokaryotes and eukaryotes. The second last step is catalysed by 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase EC:2.1.2.3 (AICARFT), this enzyme catalyses the formylation of AICAR

with 10-formyl-tetrahydrofolate to yield FAICAR and tetrahydrofolate [1]. The last step is catalysed by IMP (Inosine monophosphate) cyclohydrolase EC:3.5.4.10 (IMPCase), cyclizing FAICAR (5-formylaminoimidazole-4-carboxamide ribonucleotide) to IMP [1].

5 Number of members: 22

[1] Akira T, Komatsu M, Nango R, Tomooka A, Konaka K, Yamauchi M, Kitamura Y, Nomura S, Tsukamoto I; Medline: 97473523 Molecular cloning and expression of a rat cDNA encoding 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase" [published erratum appears in Gene 1998 Feb 27;208(2):337] Gene 1997;197:289-293.

[2] Rayl EA, Moroson BA, Beardsley GP; Medline: 96147205 The human purH gene product, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase. Cloning, sequencing, expression, purification, kinetic analysis, and domain mapping." J Biol Chem 1996;271:2225-2233.

833. (AOX)

Alternative oxidase

The alternative oxidase is used as a second terminal oxidase in the mitochondria, electrons are transferred directly from reduced ubiquinol to oxygen forming water [2]. This is not coupled to ATP synthesis and is not inhibited by cyanide, this pathway is a single step process [1]. In rice the transcript levels of the alternative oxidase are increased by low temperature [1].

Number of members: 27

[1] Ito Y, Saisho D, Nakazono M, Tsutsumi N, Hirai A; Medline: 98086211 Transcript levels of tandem-arranged alternative oxidase genes in rice are increased by low temperature." Gene 1997;203:121-129.

[2] Li Q, Ritzel RG, McLean LL, McIntosh L, Ko T, Bertrand H, Nargang FE; Medline: 96366413 Cloning and analysis of the alternative oxidase gene of *Neurospora crassa*." Genetics 1996;142:129-140.

5

834. (APH)

Protein kinases signatures and profile

Cross-reference(s): PS00107; PROTEIN_KINASE_ATP, PS00108;

10 PROTEIN_KINASE_ST, PS00109; PROTEIN_KINASE_TYR, PS50011;
PROTEIN_KINASE_DOM

Eukaryotic protein kinases [1 to 5] are enzymes that belong to a very extensive family of proteins which share a conserved catalytic core common to both serine/threonine and tyrosine
15 protein kinases. There are a number of conserved regions in the catalytic domain of protein kinases. Two of these regions have been selected to build signature patterns. The first region, which is located in the N-terminal extremity of the catalytic domain, is a glycine-rich stretch of residues in the vicinity of a lysine residue, which has been shown to be involved in ATP binding. The second region, which is located in the central part of the catalytic domain,
20 contains a conserved aspartic acid residue which is important for the catalytic activity of the enzyme [6]; two signature patterns were derived for that region: one specific for serine/threonine kinases and the other for tyrosine kinases. A profile was developed which is based on the alignment in [1] and covers the entire catalytic domain.

25 Consensus pattern: [LIV]-G-{P}-G-{P}-[FYWMGSTNH]-[SGA]-[PW]-[LIVCAT]-{PD}-x-[GSTACLIVMFY]-x(5,18)-[LIVMFYWCSTAR]-[AIVP]-[LIVMFAGCKR]-K [K binds ATP]

30 Sequences known to belong to this class detected by the pattern the majority of known protein kinases but it fails to find a number of them, especially viral kinases which are quite divergent in this region and are completely missed by this pattern.

Consensus pattern: [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3) [D is an active site residue]

Sequences known to belong to this class detected by the pattern. Most serine/ threonine specific protein kinases with 10 exceptions (half of them viral kinases) and also Epstein-Barr virus BGLF4 and *Drosophila ninaC* which have respectively Ser and Arg instead of the conserved Lys and which are therefore detected by the tyrosine kinase specific pattern described below.

Consensus pattern: [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-[RSTAC]-x(2)-N-[LIVMFYC](3) [D is an active site residue] tyrosine specific protein kinases with the exception of human ERBB3 and mouse blk. This pattern will also detect most bacterial aminoglycoside phosphotransferases [8,9] and herpesviruses ganciclovir kinases [10]; which are proteins structurally and evolutionary related to protein kinases. Sequences known to belong to this class detected by the profile ALL, except for three viral kinases. This profile also detects receptor guanylate cyclases (see <PDOC00430>) and 2-5A-dependent ribonucleases. Sequence similarities between these two families and the eukaryotic protein kinase family have been noticed before. It also detects *Arabidopsis thaliana* kinase- like protein TMKL1 which seems to have lost its catalytic activity.

Note if a protein analyzed includes the two protein kinase signatures, the probability of it being a protein kinase is close to 100%. Note eukaryotic-type protein kinases have also been found in prokaryotes such as *Myxococcus xanthus* [11] and *Yersinia pseudotuberculosis*. Note the patterns shown above has been updated since their publication in [7]. Note this documentation entry is linked to both signature patterns and a profile. As the profile is much more sensitive than the patterns, you should use it if you have access to the necessary software tools to do so.

References

- [1] Hanks S.K., Hunter T., FASEB J. 9:576-596(1995).
- [2] Hunter T., Meth. Enzymol. 200:3-37(1991).
- [3] Hanks S.K., Quinn A.M., Meth. Enzymol. 200:38-62(1991).
- [4] Hanks S.K., Curr. Opin. Struct. Biol. 1:369-383(1991).
- [5] Hanks S.K., Quinn A.M., Hunter T., Science 241:42-52(1988).
- [6] Knighton D.R., Zheng J., Ten Eyck L.F., Ashford V.A., Xuong N.-H., Taylor, S.S., Sowadski J.M., Science 253:407-414(1991).

[7] Bairoch A., Claverie J.-M., Nature 331:22(1988).

[8] Benner S., Nature 329:21-21(1987).

[9] Kirby R., J. Mol. Evol. 30:489-492(1992).

[10] Littler E., Stuart A.D., Chee M.S., Nature 358:160-162(1992).

5 [11] Munoz-Dorado J., Inouye S., Inouye M., Cell 67:995-1006(1991).

835. (Asp_Glu_race)

Aspartate and glutamate racemases signatures

10

Cross-reference(s) PS00923; ASP_GLU_RACEMASE_1 PS00924;

ASP_GLU_RACEMASE_2

Aspartate racemase (EC 5.1.1.13) and glutamate racemase (EC 5.1.1.3) are two evolutionary
15 related bacterial enzymes that do not seem to require a cofactor for their activity [1].

Glutamate racemase, which interconverts L-glutamate into D-glutamate, is required for the
biosynthesis of peptidoglycan and some peptide-based antibiotics such as gramicidin S. In
addition to characterized aspartate and glutamate racemases, this family also includes a
hypothetical protein from *Erwinia carotovora* and one from *Escherichia coli* (*ygeA*). Two
20 conserved cysteines are present in the sequence of these enzymes. They are expected to play
a role in catalytic activity by acting as bases in proton abstraction from the substrate.

Signature patterns were developed for both cysteines.

Consensus pattern: [IVA]-[LIVM]-x-C-x(0,1)-N-[ST]-[MSA]-[STH]-[LIVFYSTANK]

25

Consensus pattern: [LIVM](2)-x-[AG]-C-T-[DEH]-[LIVMFY]-[PNGRS]-x-[LIVM]

[1] Gallo K.A., Knowles J.R., Biochemistry 32:3981-3990(1993).

30

836. (ATP-sulfurylase)

ATP-sulfurylase

This family consists of ATP-sulfurylase or sulfate adenyltransferase EC:2.7.7.4 some of which are part of a bifunctional polypeptide chain associated with adenosyl phosphosulphate (APS) kinase APS_kinase. Both enzymes are required for PAPS (phosphoadenosine-phosphosulfate) synthesis from inorganic sulphate [2]. ATP sulfurylase catalyses the synthesis of adenosine-phosphosulfate APS from ATP and inorganic sulphate [1].

Number of members: 37

[1] Kurima K, Warman ML, Krishnan S, Domowicz M, Krueger RC Jr, Deyrup A, Schwartz NB; Medline: 98337975 A member of a family of sulfate-activating enzymes causes murine brachymorphism" [published erratum appears in Proc Natl Acad Sci U S A 1998 Sep 29;95(20):12071] Proc Natl Acad Sci U S A 1998;95:8681-8685.

[2] Rosenthal E, Leustek T; Medline: 96096529 A multifunctional Urechis caupo protein, PAPS synthetase, has both ATP sulfurylase and APS kinase activities." Gene 1995;165:243-248.

837. (ATP-synt_F)

ATP synthase (F/14-kDa) subunit

This family includes 14-kDa subunit from vATPases [1], which is in the peripheral catalytic part of the complex [2]. The family also includes archaebacterial ATP synthase subunit F [3].

Number of members: 23

[1] Guo Y, Kaiser K, Wieczorek H, Dow JA; Medline: 96269411 The Drosophila melanogaster gene vha14 encoding a 14-kDa F-subunit of the vacuolar ATPase." Gene 1996;172:239-243.

[2] Peng SB, Crider BP, Tsai SJ, Xie XS, Stone DK; Medline: 96216416 Identification of a 14-kDa subunit associated with the catalytic sector of clathrin-coated vesicle H⁺-ATPase." J Biol Chem 1996;271:3324-3327.

[3] Wilms R, Freiberg C, Wegerle E, Meier I, Mayer F, Muller V; Medline: 96324968
Subunit structure and organization of the genes of the A1A0 ATPase from the Archaeon
Methanosarcina mazei Go1." J Biol Chem 1996;271:18843-18852.

5

838. (CBD_4)

Starch binding domain

Number of members: 48

10

839. (CbiX)

The function of CbiX is uncertain, however it is found in cobalamin biosynthesis operons and
so may have a related function. Some CbiX proteins contain a striking histidine-rich region at
their C-terminus, which suggests that it might be involved in metal chelation [1].

Number of members: 6

[1] Raux E, Lanois A, Warren MJ, Rambach A, Thermes C; Medline: 98416126 Cobalamin
(vitamin B12) biosynthesis: identification and characterization of a Bacillus megaterium cobI
operon." Biochem J 1998;335:159-166.

25

840. (Complex1_51K)

Respiratory-chain NADH dehydrogenase 51 Kd subunit signatures Cross-reference(s)
PS00644; COMPLEX1_51K_1 PS00645; COMPLEX1_51K_2

30

Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or
NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner
mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria
(as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this
bioenergetic enzyme complex there is one with a molecular weight of 51 Kd (in mammals),

which is the second largest subunit of complex I and is a component of the iron-sulfur (IP) fragment of the enzyme. It seems to bind to NAD, FMN, and a 2Fe-2S cluster.

The 51 Kd subunit is highly similar to [3,4]:

- 5 - Subunit alpha of *Alcaligenes eutrophus* NAD-reducing hydrogenase (gene hoxF) which also binds to NAD, FMN, and a 2Fe-2S cluster.
- Subunit NQO1 of *Paracoccus denitrificans* NADH-ubiquinone oxidoreductase.
- Subunit F of *Escherichia coli* NADH-ubiquinone oxidoreductase (gene nuoF).

- 10 The 51 Kd subunit and the bacterial hydrogenase alpha subunit contains three regions of sequence similarities. The first one most probably corresponds to the NAD-binding site, the second to the FMN-binding site, and the third one, which contains three cysteines, to the iron-sulfur binding region. Signature patterns have been developed for the FMN-binding and for the 2Fe-2S binding regions.

- 15 Consensus pattern: G-[AM]-G-[AR]-Y-[LIVM]-C-G-[DE](2)-[STA](2)-[LIM](2)-[EN]- S
Consensus pattern: E-S-C-G-x-C-x-P-C-R-x-G [The three C's are putative 2Fe-2S ligands]

- [1] Ragan C.I., Curr. Top. Bioenerg. 15:1-36(1987).
- 20 [2] Weiss H., Friedrich T., Hofhaus G., Preis D., Eur. J. Biochem. 197:563-576(1991).
- [3] Fearnley I.M., Walker J.E. Biochim. Biophys. Acta 1140:105-134(1992).
- [4] Weidner U., Geier S., Ptöck A., Friedrich T., Leif H., Weiss H., J. Mol. Biol. 233:109-122(1993).

- 25 841. (DAP_epimerase)
Diaminopimelate epimerase signature

Cross-reference(s) PS01326; DAP_EPIMERASE

- 30 Diaminopimelate epimerase (EC 5.1.1.7) catalyzes the isomerization of L,L- to D,L-meso-diaminopimelate in the biosynthetic pathway leading from aspartate to lysine. This enzyme is a protein of about 30 Kd. Two conserved cysteines seem [1] to function as the acid and base in the catalytic mechanism. As a signature pattern, the region surrounding the first of these two active site cysteines were selected.

Consensus pattern: N-x-D-G-S-x(4)-C-G-N-[GA]-x-R [C is an active site residue] Sequences known to belong to this class detected by the pattern ALL, except for an *Anabaena* dapF which has a Ser instead of the active site Cys.

5

[1] Cirilli M., Zheng R., Scapin G., Blanchard J.S., *Biochemistry* 37:16452-16458(1998).

842. (DNA_gyraseB_C)

10 DNA topoisomerase II signature

Cross-reference(s) PS00177; TOPOISOMERASE_II

DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type II topoisomerases are ATP-dependent and act by passing a DNA segment through a transient double-strand break. Topoisomerase II is found in phages, archaeobacteria, prokaryotes, eukaryotes, and in African Swine Fever virus (ASF). In bacteriophage T4 topoisomerase II consists of three subunits (the product of genes 39, 52 and 60). In prokaryotes and in archaeobacteria the enzyme, known as DNA gyrase, consists of two subunits (genes *gyrA* and *gyrB* [E2]). In some bacteria, a second type II topoisomerase has been identified; it is known as topoisomerase IV and is required for chromosome segregation, it also consists of two subunits (genes *parC* and *parE*). In eukaryotes, type II topoisomerase is a homodimer.

25 There are many regions of sequence homology between the different subtypes of topoisomerase II. The relation between the different subunits is shown in the following representation:

<-----About-1400-residues----->

30 [-----Protein 39-*-----][---Protein 52----] Phage T4
 [-----gyrB-----*-----][-----gyrA-----] Prokaryote II
 Archaeobacteria
 [-----parE-----*-----][-----parD-----] Prokaryote IV
 [-----*-----] Eukaryote and

ASF

*: Position of the pattern.

5 As a signature pattern for this family of proteins, a region that contains a highly conserved pentapeptide was selected. The pattern is located in *gyrB*, in *parE*, and in protein 39 of phage T4 topoisomerase.

Consensus pattern: [LIVMA]-x-E-G-[DN]-S-A-x-[STAG]

- 10 [1] Sternglanz R., Curr. Opin. Cell Biol. 1:533-535(1990).
 [2] Bjornsti M.-A., Curr. Opin. Struct. Biol. 1:99-103(1991).
 [3] Sharma A., Mondragon A., Curr. Opin. Struct. Biol. 5:39-47(1995).
 [4] Roca J., Trends Biochem. Sci. 20:156-160(1995).

15 843. (DUF16)

Protein of unknown function

The function of this protein is unknown. It appears to only occur in *Mycoplasma pneumoniae*.

Number of members: 26

[1] Himmelreich R, Hilbert H, Plagens H, Pirkel E, Li BC, Herrmann R; Medline: 97105885
 25 Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*.
 Nucleic Acids Res 1996;24:4420-4449.

844. (DUF21)

30 Domain of unknown function

This transmembrane region has no known function. Many of the sequences in this family are annotated as hemolysins, however this is due to a similarity to Swiss:Q54318 that does not

contain this domain. This domain is found in the N-terminus of the proteins adjacent to two intracellular CBS domains CBS.

Number of members: 42

5

845. (DUF56)

Integral membrane protein

10

The members of this family are putative integral membrane proteins. The function of the family is unknown, however the family includes Sec59 from yeast. Sec59 is a dolichol kinase EC:2.7.1.108, but it is not clear if the enzymatic activity resides in this region or its N terminal region.

15

Number of members: 13

846. (DUF94)

20

Domain of unknown function

The function of this domain is unknown. It is found in both eukaryotes and archaeobacteria. The alignment contains a completely conserved aspartate residue that may be functionally important. The eukaryotic domains contains three conserved cysteines and a histidine that might be metal binding, however these are absent in the archaeobacterial proteins.

25

Number of members: 9

30

847. (FF)

FF domain

This domain may be involved in protein-protein interaction [1].

Number of members: 42

- 5 [1] Bedford MT, Leder P; Medline: 99322199 The FF domain: a novel motif that often accompanies WW domains." Trends Biochem Sci 1999;24:264-265.

848. (FLO_LFY)

10 Floricaula / Leafy protein

This family consists of various plant development proteins which are homologues of floricaula (FLO) and Leafy (LFY) proteins which are floral meristem identity proteins. Mutations in the sequences of these proteins affect flower and leaf development.

15 Number of members: 16

- [1] Hofer J, Turner L, Hellens R, Ambrose M, Matthews P, Michael A, Ellis N; Medline: 97411151 UNIFOLIATA regulates leaf and flower morphogenesis in pea." Curr Biol 1997;7:581-587.

- 20 [2] Weigel D, Alvarez J, Smyth DR, Yanofsky MF, Meyerowitz EM; Medline: 92274452 LEAFY controls floral meristem identity in Arabidopsis." Cell 1992;69:843-859.

25 849. (G-patch)

G-patch domain

This domain is found in a number of RNA binding proteins, and is also found in proteins that contain RNA binding domains. This suggests that this domain may have an RNA binding function. This domain has seven highly conserved glycines.

30 Number of members: 47

[1] Aravind L, Koonin EV; Medline: 10470032 G-patch: a new conserved domain in eukaryotic RNA-processing proteins and type D retroviral polyproteins." Trends Biochem Sci 1999;24:342-344.

5

850. (Gram-ve_porins)

General diffusion Gram-negative porins signature

Cross-reference(s) PS00576; GRAM_NEG_PORIN

- 10 The outer membrane of Gram-negative bacteria acts as a molecular filter for hydrophilic compounds. Proteins, known as porins [1], are responsible for the 'molecular sieve' properties of the outer membrane. Porins form large water- filled channels which allows the diffusion of hydrophilic molecules into the periplasmic space. Some porins form general diffusion channels that allows any solutes up to a certain size (that size is known as the exclusion limit)
- 15 to cross the membrane, while other porins are specific for a solute and contain a binding site for that solute inside the pores (these are known as selective porins). As porins are the major outer membrane proteins, they also serve as receptor sites for the binding of phages and bacteriocins. General diffusion porins generally assemble as trimer in the membrane and the transmembrane core of these proteins is composed exclusively of beta strands [2]. It has been
- 20 shown [3] that a number of general porins are evolutionary related, these porins are:
- Enterobacteria phoE.
 - Enterobacteria ompC.
 - Enterobacteria ompF.
 - Enterobacteria nmpC.
- 25 - Bacteriophage PA-2 LC.
- Neisseria PI.A.
 - Neisseria PI.B.

30

As a signature pattern a conserved region was selected, located in the C-terminal part of these proteins, which spans two putative transmembrane beta strands.

Consensus pattern: [LIVMFY]-x(2)-G-x(2)-Y-x-F-x-K-x(2)-[SN]-[STAV]-[LIVMFYW]- V

[1] Benz R., Bauer K., Eur. J. Biochem. 176:1-19(1988).

[2] Jap B.K., Walian P.J., Q. Rev. Biophys. 23:367-403(1990).

[3] Jeanteur D., Lakey J.H., Pattus F., Mol. Microbiol. 5:2153-2164(1991).

5 851. (HlyD)

HlyD family secretion proteins signature

Cross-reference(s) PS00543; HLYD_FAMILY

Gram-negative bacteria produce a number of proteins which are secreted into the growth medium by a mechanism that does not require a cleaved N-terminal signal sequence. These proteins, while having different functions, require the help of two or more proteins for their secretion across the cell envelope. Amongst which a protein belonging to the ABC transporters family (see the relevant entry <PDOC00185>) and a protein belonging to a family which is currently composed [1 to 5] of the following members:

15	Gene	Species	Protein which is exported
	-----	-----	-----
	hlyD	Escherichia coli	Hemolysin
	appD	A.pleuropneumoniae	Hemolysin
	lcnD	Lactococcus lactis	Lactococcin A
20	lktD	A.actinomycetemcomitans	Leukotoxin
		Pasteurella haemolytica	
	rtxD	A.pleuropneumoniae	Toxin-III
	cyaD	Bordetella pertussis	Calmodulin-sensitive adenylate cyclase-hemolysin (cyclolysin)
25	cvaA	Escherichia coli	Colicin V
	prtE	Erwinia chrysanthemi	Extracellular proteases B and C
	aprE	Pseudomonas aeruginosa	Alkaline protease
	emrA	Escherichia coli	Drugs and toxins
	yjcR	Escherichia coli	Unknown

30 These proteins are evolutionary related and consist of from 390 to 480 amino acid residues. They seem to be anchored in the inner membrane by a N-terminal transmembrane region. Their exact role in the secretion process is not yet known. The C-terminal section of these proteins is the best conserved region; a signature pattern from that region was derived.

Consensus pattern: [LIVM]-x(2)-G-[LM]-x(3)-[STGAV]-x-[LIVMT]-x-[LIVMT]-[GE]-x-[KR]-x-[LIVMFYW](2)-x-[LIVMFYW](3)

Sequences known to belong to this class detected by the pattern ALL, except for *emrA* and *yjcR*.

5

References:

[1] Gilson L., Mahanty H.K., Kolter R., EMBO J. 9:3875-3884(1990).

[2] Letoffe S., Delepelaire P., Wandersman C., EMBO J. 9:1375-1382(1990).

[3] Stoddard G.W., Petzel J.P., van Belkum M.J., Kok J., McKay L.L., Appl. Environ.

10 Microbiol. 58:1952-1961(1992).

[4] Duong F., Lazdunski A., Cami B., Murgier M., Gene 121:47-54(1992).

[5] Lewis K., Trends Biochem. Sci. 19:119-123(1994).

15 852. (IBR)

In Between Ring fingers

The IBR (In Between Ring fingers) domain is found to occur between pairs of ring fingers (zf-C3HC4). The function of this domain is unknown. This domain has also been called the

20 C6HC domain and DRIL (for double RING finger linked) domain [2].

Number of members: 25

[1] Morett E, Bork P; Medline: 10366851 A novel transactivation domain in parkin."Trends Biochem Sci 1999;24:229-231.

25 [2] van der Reijden BA, Erpelinck-Verschueren CA, Lowenberg B, Jansen JH; Medline: 99349709 TRIADS: a new class of proteins with a novel cysteine-rich signature." Protein Sci 1999;8:1557-1561.

30 853. (IPPT)

IPP transferase

[1] Durand JM, Bjork GR, Kuwae A, Yoshikawa M, Sasakawa C; Medline: 97440126 The modified nucleoside 2-methylthio-N6-isopentenyladenosine in tRNA of *Shigella flexneri* is required for expression of virulence genes." J Bacteriol 1997;179:5777-5782.

[2] Boguta M, Hunter LA, Shen WC, Gillman EC, Martin NC, Hopper AK; Medline:

5 94187700 Subcellular locations of MOD5 proteins: mapping of sequences sufficient for targeting to mitochondria and demonstration that mitochondrial and nuclear isoforms commingle in the cytosol." Mol Cell Biol 1994;14:2298-2306.

[3] Gillman EC, Slusher LB, Martin NC, Hopper AK; Medline: 91203856 MOD5 translation initiation sites determine N6-isopentenyladenosine modification of mitochondrial and cytoplasmic tRNA." Mol Cell Biol 1991;11:2382-2390.

854. (KE2)

KE2 family protein

15 The function of members of this family is unknown, although they have been suggested to contain a DNA binding leucine zipper motif [2].

Number of members: 9

20 [1] Ha H, Abe K, Artzt K; Medline: 92084131 Primary structure of the embryo-expressed gene KE2 from the mouse H-2K region." Gene 1991;107:345-346.

[2] Shang HS, Wong SM, Tan HM, Wu M; Medline: 95129859 YKE2, a yeast nuclear gene encoding a protein showing homology to mouse KE2 and containing a putative leucine-zipper motif." Gene 1994;151:197-201.

855. (Lipoprotein_6)

Prokaryotic membrane lipoprotein lipid attachment site

Cross-reference(s) PS00013; PROKAR_LIPOPROTEIN

In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which is cleaved by a specific lipoprotein signal peptidase (signal peptidase II). The peptidase recognizes a conserved sequence and cuts upstream of a cysteine residue to which

a glyceride-fatty acid lipid is attached [1]. Some of the proteins known to undergo such processing currently include (for recent listings see [1,2,3]):

- Major outer membrane lipoprotein (murein-lipoproteins) (gene lpp).
- Escherichia coli lipoprotein-28 (gene nlpA).
- 5 - Escherichia coli lipoprotein-34 (gene nlpB).
- Escherichia coli lipoprotein nlpC.
- Escherichia coli lipoprotein nlpD.
- Escherichia coli osmotically inducible lipoprotein B (gene osmB).
- Escherichia coli osmotically inducible lipoprotein E (gene osmE).
- 10 - Escherichia coli peptidoglycan-associated lipoprotein (gene pal).
- Escherichia coli rare lipoproteins A and B (genes rplA and rplB).
- Escherichia coli copper homeostasis protein cutF (or nlpE).
- Escherichia coli plasmids traT proteins.
- Escherichia coli Col plasmids lysis proteins.
- 15 - A number of Bacillus beta-lactamases.
- Bacillus subtilis periplasmic oligopeptide-binding protein (gene oppA).
- Borrelia burgdorferi outer surface proteins A and B (genes ospA and ospB).
- Borrelia hermsii variable major protein 21 (gene vmp21) and 7 (gene vmp7).
- Chlamydia trachomatis outer membrane protein 3 (gene omp3).
- 20 - Fibrobacter succinogenes endoglucanase cel-3.
- Haemophilus influenzae proteins Pal and Pcp.
- Klebsiella pullulunase (gene pulA).
- Klebsiella pullulunase secretion protein pulS.
- Mycoplasma hyorhinitis protein p37.
- 25 - Mycoplasma hyorhinitis variant surface antigens A, B, and C (genes vlpABC).
- Neisseria outer membrane protein H.8.
- Pseudomonas aeruginosa lipopeptide (gene lppL).
- Pseudomonas solanacearum endoglucanase egl.
- Rhodopseudomonas viridis reaction center cytochrome subunit (gene cytC).
- 30 - Rickettsia 17 Kd antigen.
- Shigella flexneri invasion plasmid proteins mxiJ and mxiM.
- Streptococcus pneumoniae oligopeptide transport protein A (gene amiA).
- Treponema pallidum 34 Kd antigen.
- Treponema pallidum membrane protein A (gene tmpA).

- *Vibrio harveyi* chitobiase (gene chb).
- *Yersinia* virulence plasmid protein yscJ.
- Halocyanin from *Natrobacterium pharaonis* [4], a membrane associated copper-binding protein. This is the first archaeobacterial protein known to be modified in such a fashion).

5

From the precursor sequences of all these proteins, a consensus pattern and a set of rules to identify this type of post-translational modification were derived.

Consensus pattern: {DERK}(6)-[LIVMFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1)

10

The cysteine must be between positions 15 and 35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the first seven positions of the sequence. Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROT some 100 prokaryotic proteins. Some of them are not membrane lipoproteins, but at least half of them could be.

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References

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- [1] Hayashi S., Wu H.C., J. Bioenerg. Biomembr. 22:451-471(1990).
- [2] Klein P., Somorjai R.L., Lau P.C.K., Protein Eng. 2:15-20(1988).
- [3] von Heijne G., Protein Eng. 2:531-534(1989).
- [4] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

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856. (Lipoprotein_7)
Adhesin lipoprotein

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This family consists of the p50 and variable adherence-associated antigen (Vaa) adhesins from *Mycoplasma hominis*. *M. hominis* is a mycoplasma associated with human urogenital diseases, pneumonia, and septic arthritis [1]. An adhesin is a cell surface molecule that mediates adhesion to other cells or to the surrounding surface or substrate. The Vaa antigen is a 50-kDa surface lipoprotein that has four tandem repetitive DNA sequences encoding a periodic peptide structure, and is highly immunogenic in the human host [1]. p50 is also a 50-

kDa lipoprotein, having three repeats A,B and C, that may be a tetramer of 191-kDa in its native environment [2].

Number of members: 18

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[1] Zhang Q, Wise KS; Medline: 96294788 Molecular basis of size and antigenic variation of a *Mycoplasma hominis* adhesin encoded by divergent vaa genes. *Infect Immun* 1996;64:2737-2744.

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[2] Henrich B, Kitzew A, Feldmann RC, Schaal H, Hadding U; Medline: 97047675 Repetitive elements of the *Mycoplasma hominis* adhesin p50 can be differentiated by monoclonal antibodies." *Infect Immun* 1996;64:4027-4034.

857. (MaoC_like)

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MaoC like domain

The MaoC protein is found to share similarity with a wide variety of enzymes; estradiol 17 beta-dehydrogenase 4, peroxisomal hydratase-dehydrogenase-epimerase, fatty acid synthase beta subunit. All these enzymes contain other domains. This domain is also present in the

20 NodN nodulation protein N. No specific function has been assigned to this region of any of these proteins. The maoC gene is part of a operon with maoA which is involved in the synthesis of monoamine oxidase [1].

Number of members: 46

25

[1] Sugino H, Sasaki M, Azakami H, Yamashita M, Murooka Y Medline: 96235221 A monoamine-regulated *Klebsiella aerogenes* operon containing the monoamine oxidase structural gene (maoA) and the maoC gene." *J Bacteriol* 1992;174:2485-2492.

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858. (MSP)

Manganese-stabilizing protein / photosystem II polypeptide

This family consists of the 33 KDa photosystem II polypeptide from the oxygen evolving complex (OEC) of plants and cyanobacteria. The protein is also known as the manganese-stabilizing protein as it is associated with the manganese complex of the OEC and may provide the ligands for the complex [1].

5

Number of members: 17

[1] Philbrick JB, Zilinskas BA; Medline: 88334494 "Cloning, nucleotide sequence and mutational analysis of the gene encoding the Photosystem II manganese-stabilizing polypeptide of *Synechocystis* 6803." Mol Gen Genet 1988;212:418-425.

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859. (NAC)

15 [1] Makarova KS, Aravind L, Galperin MY, Grishin NV, Tatusov RL, Wolf YI, Koonin EV; Medline: 99342100 Comparative genomics of the Archaea (Euryarchaeota): evolution of conserved protein families, the stable core, and the variable shell." Genome Res 1999;9:608-628.

20 Number of members: 27

860. (Nop)

Putative snoRNA binding domain

25

This family consists of various Pre RNA processing ribonucleoproteins. The function of the aligned region is unknown however it may be a common RNA or snoRNA or Nop1p binding domain. Nop5p (Nop58p) Swiss:Q12499 from yeast is the protein component of a ribonucleoprotein protein required for pre-18s rRNA processing and is suggested to function with Nop1p in a snoRNA complex [1]. Nop56p Swiss:O00567 and Nop5p interact with Nop1p and are required for ribosome biogenesis [2]. Prp31p Swiss:p49704 is required for pre-mRNA splicing in *S. cerevisiae* [3].

30

Number of members: 23

[1] Wu P, Brockenbrough JS, Metcalfe AC, Chen S, Aris JP; Medline: 98298165 Nop5p is a small nucleolar ribonucleoprotein component required for pre- 18 S rRNA processing in yeast." J Biol Chem 1998;273:16453-16463.

[2] Gautier T, Berges T, Tollervey D, Hurt E; Medline: 8038777 Nucleolar KKE/D repeat proteins Nop56p and Nop58p interact with Nop1p and are required for ribosome biogenesis." Mol Cell Biol 1997;17:7088-7098.

[3] Weidenhammer EM, Singh M, Ruiz-Noriega M, Woolford JL Jr; Medline: 96184869

The PRP31 gene encodes a novel protein required for pre-mRNA splicing in *Saccharomyces cerevisiae*." Nucleic Acids Res 1996;24:1164-1170.

861. (Nramp)

Natural resistance-associated macrophage protein

The natural resistance-associated macrophage protein (NRAMP) family consists of Nramp1, Nramp2, and yeast proteins Smf1 and Smf2. The NRAMP family is a novel family of functional related proteins defined by a conserved hydrophobic core of ten transmembrane domains [5]. This family of membrane proteins are divalent cation transporters. Nramp1 is an integral membrane protein expressed exclusively in cells of the immune system and is recruited to the membrane of a phagosome upon phagocytosis [1]. By controlling divalent cation concentrations Nramp1 may regulate the interphagosomal replication of bacteria [1]. Mutations in Nramp1 may genetically predispose an individual to susceptibility to diseases including leprosy and tuberculosis conversely this might however provide protection form rheumatoid arthritis [1]. Nramp2 is a multiple divalent cation transporter for Fe2+, Mn2+ and Zn2+ amongst others it is expressed at high levels in the intestine; and is major transferrin-independent iron uptake system in mammals [1]. The yeast proteins Smf1 and Smf2 may also transport divalent cations [3].

Number of members: 36

[1] Govoni G, Gros P; Medline: 98383996 Macrophage NRAMP1 and its role in resistance to microbial infections." Inflamm Res 1998;47:277-284.

[2] Agronoff DD, Krishna S Medline: 98294035 Metal ion homeostasis and intracellular parasitism." Mol Microbiol 1998;28:403-412.

[3] Pinner E, Gruenheid S, Raymond M, Gros P; Medline: 98030569 Functional complementation of the yeast divalent cation transporter family SMF by NRAMP2, a member of the mammalian natural resistance- associated macrophage protein family." J Biol Chem 1997;272:28933-28938.

[4] Cellier M, Belouchi A, Gros P; Medline: 96402487 Resistance to intracellular infections: comparative genomic analysis of Nramp." Trends Genet 1996;12:201-204.

[5] Cellier M, Prive G, Belouchi A, Kwan T, Rodrigues V, Chia W, Gros P; Medline: 96036029 Nramp defines a family of membrane proteins." Proc Natl Acad Sci U S A 1995;92:10089-10093.

862. (NTP_transf_2)

Nucleotidyltransferase domain

Members of this family belong to a large family of nucleotidyltransferases [1].

Number of members: 83

[1] Holm L, Sander C; Medline: 96005605 DNA polymerase beta belongs to an ancient nucleotidyltransferase superfamily." Trends Biochem Sci 1995;20:345-347.

863. (Paramyxo_P)

Paramyxovirus P phosphoprotein

This family consists of paramyxovirus P phosphoprotein from sendai virus and human and bovine parainfluenza viruses. The P protein is an essential part of the viral RNA polymerase complex formed from the P and L proteins [1]. The exact role of the P protein in this complex is unknown but it is involved in multiple protein-protein interactions and binding the polymerase complex to the nucleocapsid or ribonucleoprotein template [1]. It also appears to be important for the proper folding of the L protein [1]. The paramyxoviruses have a negative sense ssRNA genome [1].

Number of members: 15

[1] Bowman MC, Smallwood S, Moyer SA; Medline: 99329169 Dissection of Individual Functions of the Sendai Virus Phosphoprotein in Transcription." J Virol 1999;73:6474-6483.
 [2] Matsuoka Y, Curran J, Pelet T, Kolakofsky D, Ray R, Compans RW; Medline: 91237868 The P gene of human parainfluenza virus type 1 encodes P and C proteins but not a cysteine-rich V protein." J Virol 1991;65:3406-3410.

864. (Patatin)

This family consists of various patatin glycoproteins from plants. The patatin protein accounts for up to 40% of the total soluble protein in potato tubers [2]. Patatin is a storage protein but it also has the enzymatic activity of lipid acyl hydrolase, catalysing the cleavage of fatty acids from membrane lipids [2].

Number of members: 21

[1] Banfalvi Z, Kostyal Z, Barta E; Medline: 95107249 Solanum brevidens possesses a non-sucrose-inducible patatin gene." Mol Gen Genet 1994;245:517-522.
 [2] Mignery GA, Pikaard CS, Park WD; Medline: 88226014 Molecular characterization of the patatin multigene family of potato." Gene 1988;62:27-44.

865. (Pentapeptide_2)

Pentapeptide repeats (8 copies)

These repeats are found in many mycobacterial proteins. These repeats are most common in the PPE family of proteins, where they are found in the MPTR subfamily of PPE proteins. The function of these repeats is unknown. The repeat can be approximately described as XNXGX, where X can be any amino acid. These repeats are similar to Pentapeptide [1], however it is not clear if these two families are structurally related.

Number of members: 362

[1] Bateman A, Murzin A, Teichmann SA; Medline: 98318059 Structure and distribution of pentapeptide repeats in bacteria." Protein Sci 1998;7:1477-1480.

[2] Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Barrell BG; Medline: 98295987 Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence." Nature 1998;393:537-544.

866. (Peptidase_C13)

Peptidase C13 family

This family of peptidases is known as the hemoglobinase family because it contains a globin degrading enzyme from blood parasites Swiss:P42665. However relatives are found in plants and other organisms that have other functions. Members of this family are asparaginyl peptidases [1].

Number of members: 26

[1] Chen JM, Dando PM, Rawlings ND, Brown MA, Young NE, Stevens RA, Hewitt E, Watts C, Barrett AJ; Medline: 97218252 Cloning, isolation, and characterization of mammalian legumain, an asparaginyl endopeptidase." J Biol Chem 1997;272:8090-8098.

867. (Pro_dh)

Proline dehydrogenase

Number of members: 25

[1] Ling M, Allen SW, Wood JM; Medline: 95055736 Sequence analysis identifies the proline dehydrogenase and delta 1- pyrroline-5-carboxylate dehydrogenase domains of the multifunctional *Escherichia coli* PutA protein." J Mol Biol 1994;243:950-956.

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868. (PsbP)

This family consists of the 23 kDa subunit of oxygen evolving system of photosystem II or PsbP from various plants (where it is encoded by the nuclear genome) and Cyanobacteria.

- 10 The 23 KDa PsbP protein is required for PSII to be fully operational in vivo, it increases the affinity of the water oxidation site for Cl- and provides the conditions required for high affinity binding of Ca2+ [2].

Number of members: 25

15

[1] Rova EM, Mc Ewen B, Fredriksson PO, Styring S; Medline: 97067138 Photoactivation and photoinhibition are competing in a mutant of *Chlamydomonas reinhardtii* lacking the 23-kDa extrinsic subunit of photosystem II." J Biol Chem 1996;271:28918-28924.

- 20 [2] Kochhar A, Khurana JP, Tyagi AK; Medline: 97191538 Nucleotide sequence of the psbP gene encoding precursor of 23-kDa polypeptide of oxygen-evolving complex in *Arabidopsis thaliana* and its expression in the wild-type and a constitutively photomorphogenic mutant." DNA Res 1996;3:277-285.

25 869. (PUA)

The PUA domain named after PseudoUridine synthase and Archaeosine transglycosylase, was detected in archaeal and eukaryotic pseudouridine synthases, archaeal archaeosine synthases, a family of predicted ATPases that may be involved in RNA modification, a

- 30 family of predicted archaeal and bacterial rRNA methylases. Additionally, the PUA domain was detected in a family of eukaryotic proteins that also contain a domain homologous to the translation initiation factor eIF1/SUI1; these proteins may comprise a novel type of translation factors. Unexpectedly, the PUA domain was detected also in bacterial and yeast glutamate kinases; this is compatible with the demonstrated role of these enzymes in the

regulation of the expression of other genes [1]. It is predicted that the PUA domain is an RNA binding domain.

Number of members: 48

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[1] Aravind L, Koonin EV; Medline: 99193178 Novel predicted RNA-binding domains associated with the translation machinery." J Mol Evol 1999;48:291-302.

10 870. (RF1)

eRF1-like proteins

Members of this family are peptide chain release factors. The eukaryotic Release Factor 1 proteins (eRF1s) are involved in termination of translation. The eRF1 protein is functional for all stop codons and appears to abolish read-through of these codons. This family also includes other proteins for which the precise molecular function is unknown. Many of them are from Archaeobacteria. These proteins may also be involved in translation termination but this awaits experimental verification. Number of members: 25

- 20 [1] Frolova L, Le Goff X, Rasmussen HH, Cheperegin S, Drugeon G, Kress M, Arman I, Haenni AL, Celis JE, Philippe M, et al; Medline: 95082951 A highly conserved eukaryotic protein family possessing properties of polypeptide chain release factor" [see comments] Nature 1994;372:701-703.
- [2] Drugeon G, Jean-Jean O, Frolova L, Le Goff X, Philippe M, Kisselev L, Haenni AL;
- 25 Medline: 97315314 Eukaryotic release factor 1 (eRF1) abolishes readthrough and competes with suppressor tRNAs at all three termination codons in messenger RNA." Nucleic Acids Res 1997;25:2254-2258.

30 871. (Ribosomal_L14e) Ribosomal protein L14

This family includes the eukaryotic ribosomal protein L14.

Number of members: 15

872. (Ribosomal_S27)

Ribosomal protein S27a

This family of ribosomal proteins consists mainly of the 40S ribosomal protein S27a which is synthesized as a C-terminal extension of ubiquitin (CEP). The S27a domain comprises the C-terminal half of the protein. The synthesis of ribosomal proteins as extensions of ubiquitin promotes their incorporation into nascent ribosomes by a transient metabolic stabilization and is required for efficient ribosome biogenesis [3]. The ribosomal extension protein S27a contains a basic region that is proposed to form a zinc finger; its fusion gene is proposed as a mechanism to maintain a fixed ratio between ubiquitin necessary for degrading proteins and ribosomes a source of proteins [2].

Number of members: 36

873. (Spermine_synth)

Spermine/spermidine synthase

Spermine and spermidine are polyamines. This family includes spermidine synthase that catalyses the fifth (last) step in the biosynthesis of spermidine from arginine, and spermine synthase.

Number of members: 39

[1] Mezquita J, Pau M, Mezquita C; Medline: 97449308 Characterization and expression of two chicken cDNAs encoding ubiquitin fused to ribosomal proteins of 52 and 80 amino acids." Gene 1997;195:313-319.

[2] Redman KL, Rechsteiner M; Medline: 89181932 Identification of the long ubiquitin extension as ribosomal protein S27a." Nature 1989;338:438-440.

[3] Finley D, Bartel B, Varshavsky A; Medline: 89181925 The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis." Nature 1989;338:394-401.

874. (Surp)

Surp module

[1] Denhez F, Lafyatis R; Medline: 94266805 Conservation of regulated alternative splicing and identification of functional domains in vertebrate homologs to the *Drosophila* splicing regulator, suppressor-of-white-apricot." J Biol Chem 1994;269:16170-16179.

This domain is also known as the SWAP domain. SWAP stands for Suppressor-of-White-APricot. It has been suggested that these domains may be RNA binding [1].

Number of members: 32

875. (TFIIE)

TFIIE alpha subunit

The general transcription factor TFIIE has an essential role in eukaryotic transcription initiation together with RNA polymerase II and other general factors. Human TFIIE consists of two subunits TFIIE-alpha Swiss:P29083 and TFIIE-beta Swiss:P29084 and joins the preinitiation complex after RNA polymerase II and TFIIF [1]. This family consists of the conserved amino terminal region of eukaryotic TFIIE-alpha [2] and proteins from archaeobacteria that are presumed to be TFIIE-alpha subunits also Swiss:O29501 [3].

Number of members: 12

[1] Ohkuma Y, Sumimoto H, Hoffmann A, Shimasaki S, Horikoshi M, Roeder RG; Medline: 92065982 Structural motifs and potential sigma homologies in the large subunit of human general transcription factor TFIIE." Nature 1991;354:398-401.

[2] Ohkuma Y, Hashimoto S, Roeder RG, Horikoshi M; Medline: 93087200 Identification of two large subdomains in TFIIE-alpha on the basis of homology between *Xenopus* and human sequences. Nucleic Acids Res 1992;20:5838-5838.

[3] Klenk HP, Clayton RA, Tomb JF, White O, Nelson KE, Ketchum KA, Dodson RJ, Gwinn M, Hickey EK, Peterson JD, Richardson DL, Kerlavage AR, Graham DE, Kyrpides NC, Fleischmann RD, Quackenbush J, Lee NH, Sutton GG, Gill S, Kirkness EF, Dougherty BA,

McKenney K, Adams MD, Loftus B, Venter JC, et al; Medline: 98049343 The complete genome sequence of the hyperthermophilic, sulphate- reducing archaeon *Archaeoglobus fulgidus*." *Nature* 1997;390:364-370.

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876. (Transglut_core)

Cross-reference(s) PS00547; TRANSGLUTAMINASES

- 10 Transglutaminases (EC 2.3.2.13) (TGase) [1,2] are calcium-dependent enzymes that catalyze the cross-linking of proteins by promoting the formation of isopeptide bonds between the gamma-carboxyl group of a glutamine in one polypeptide chain and the epsilon-amino group of a lysine in a second polypeptide chain. TGases also catalyze the conjugation of polyamines to proteins. The best known transglutaminase is blood coagulation factor XIII, a plasma tetrameric protein composed of two catalytic A subunits and two non-catalytic B subunits. Factor XIII is responsible for cross-linking fibrin chains, thus stabilizing the fibrin clot. Other forms of transglutaminases are widely distributed in various organs, tissues and body fluids. Sequence data is available for the following forms of TGase:
- Transglutaminase K (TGase K), a membrane-bound enzyme found in mammalian epidermis and important for the formation of the cornified cell envelope (gene TGM1).
 - Tissue transglutaminase (TGase C), a monomeric ubiquitous enzyme located in the cytoplasm (gene TGM2).
 - Transglutaminase 3, responsible for the later stages of cell envelope formation in the epidermis and the hair follicle (gene TGM3).
 - 25 - Transglutaminase 4 (gene TGM4).

A conserved cysteine is known to be involved in the catalytic mechanism of TGases. The erythrocyte membrane band 4.2 protein, which probably plays an important role in regulating the shape of erythrocytes and their mechanical properties, is evolutionary related to TGases.

- 30 However the active site cysteine is substituted by an alanine and the 4.2 protein does not show TGase activity.

Consensus pattern:[GT]-Q-[CA]-W-V-x-[SA]-[GA]-[IVT]-x(2)-T-x-[LMS]-R-[CSA]-[LV]-G [The first C is the active site residue] Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROT/NONE.

- 5 [1] Ichinose A., Bottenus R.E., Davie E.W. J. Biol. Chem. 265:13411-13414(1990).
- [2] Greenberg C.S., Birckbichler P.J., Rice R.H. FASEB J. 5:3071-3077(1991).

877. (TruB_N)

10 TruB family pseudouridylylate synthase (N terminal domain)

Members of this family are involved in modifying bases in RNA molecules. They carry out the conversion of uracil bases to pseudouridine. This family includes TruB, a pseudouridylylate synthase that specifically converts uracil 55 to pseudouridine in most tRNAs. This family
15 also includes Cbf5p that modifies rRNA [2].

Number of members: 33

[1] Nurse K, Wrzesinski J, Bakin A, Lane BG, Ofengand J; Medline: 96079944 Purification, cloning, and properties of the tRNA psi 55 synthase from Escherichia coli." RNA
20 1995;1:102-112.

[2] Lafontaine DLJ, Bousquet-Antonelli C, Henry Y, Caizergues-Ferrer M, Tollervey D; Medline: 98139521 The box H + ACA snoRNAs carry Cbf5p, the putative rRNA pseudouridine synthase." Genes Dev 1998;12:527-537.

878. (UDPGP)

UTP--glucose-1-phosphate uridylyltransferase

- 30 This family consists of UTP--glucose-1-phosphate uridylyltransferases, EC:2.7.7.9. Also known as UDP-glucose pyrophosphorylase (UDPGP) and Glucose-1-phosphate uridylyltransferase. UTP--glucose-1-phosphate uridylyltransferase catalyses the interconversion of MgUTP + glucose-1-phosphate and UDP-glucose + MgPPi [1]. UDP-glucose is an important intermediate in mammalian carbohydrate interconversion involved in

various metabolic roles depending on tissue type [1]. In Dictyostelium (slime mold) mutants in this enzyme abort the development cycle [2]. Also within the family is UDP-N-acetylglucosamine Swiss:Q16222 or AGX1 [3] and two hypothetical proteins from Borrelia burgdorferi the Lyme disease spirochaete Swiss:O51893 and Swiss:O51036.

5

Number of members: 18

[1] Duggleby RG, Chao YC, Huang JG, Peng HL, Chang HY; Medline: 96202932 Sequence differences between human muscle and liver cDNAs for UDPglucose pyrophosphorylase and kinetic properties of the recombinant enzymes expressed in Escherichia coli." Eur J Biochem 1996;235:173-179.

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[2] Ragheb JA, Dottin RP; Medline: 87231075 Structure and sequence of a UDP glucose pyrophosphorylase gene of Dictyostelium discoideum." Nucleic Acids Res 1987;15:3891-3906.

15

[3] Mio T, Yabe T, Arisawa M, Yamada-Okabe H; Medline: 98269105 The eukaryotic UDP-N-acetylglucosamine pyrophosphorylases. Gene cloning, protein expression, and catalytic mechanism. J Biol Chem 1998;273:14392-14397.

20

879. (UPF004)

Uncharacterized protein family UPF0044 signature

Cross-reference(s) PS01301; UPF0044

The following uncharacterized proteins have been shown [1] to be highly similar:

25

- Bacillus subtilis hypothetical protein yqeL.

- Escherichia coli hypothetical protein yhbY and HI1333, the corresponding Haemophilus influenzae protein.

- Methanococcus jannaschii hypothetical protein MJ0652.

These are small proteins of 10 to 15 Kd. They can be picked up in the database

30

by the following pattern. This pattern is located in the N-terminal part of these proteins.

Consensus pattern: L-[ST]-x(3)-K-x(3)-[KR]-[SGA]-x-[GA]-H-x-L-x-P-[LIV]-x(2)-[LIV]-[GA]-x(2)-G Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE.

5

880. (zf-A20)

A20-like zinc finger

A20- (an inhibitor of cell death)-like zinc fingers. The zinc finger mediates self-association in A20. These fingers also mediate IL-1-induced NF-kappa B activation.

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Number of members: 22

[1] Heyninck K, Beyaert R; Medline: 99126071 The cytokine-inducible zinc finger protein A20 inhibits IL-1-induced NF- kappaB activation at the level of TRAF6. FEBS Lett 1999;442:147-150.

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[2] De Valck D, Heyninck K, Van Crielinge W, Contreras R, Beyaert R, Fiers W; Medline: 96390831 A20, an inhibitor of cell death, self-associates by its zinc finger domain." FEBS Lett 1996;384:61-64.

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[3] Song HY, Rothe M, Goeddel DV; Medline: 96270609 The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-kappaB activation. Proc Natl Acad Sci U S A 1996;93:6721-6725.

[4] Opipari AW Jr, Boguski MS, Dixit VM; Medline: 90368626 The A20 cDNA induced by tumor necrosis factor alpha encodes a novel type of zinc finger protein." J Biol Chem 1990;265:14705-14708.

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881. (zf-PARP)

Poly(ADP-ribose) polymerase zinc finger domain

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Cross-reference(s) PS00347; PARP_ZN_FINGER_1 PS50064; PARP_ZN_FINGER_2

Poly(ADP-ribose) polymerase (EC 2.4.2.30) (PARP) [1,2] is a eukaryotic enzyme that catalyzes the covalent attachment of ADP-ribose units from NAD(+) to various nuclear

acceptor proteins. This post-translational modification of nuclear proteins is dependent on DNA. It appears to be involved in the regulation of various important cellular processes such as differentiation, proliferation and tumor transformation as well as in the regulation of the molecular events involved in the recovery of the cell from DNA damage.

Structurally, PARP, about 1000 amino-acids residues long, consists of three distinct domains: an N-terminal zinc-dependent DNA-binding domain, a central automodification domain and a C-terminal NAD-binding domain. The DNA-binding region contains a pair of zinc finger domains which have been shown to bind DNA in a zinc-dependent manner. The zinc finger domains of PARP seem to bind specifically to single-stranded DNA. DNA ligase III [3] contains, in its N-terminal section, a single copy of a zinc finger highly similar to those of PARP.

Consensus pattern: C-[KR]-x-C-x(3)-I-x-K-x(3)-[RG]-x(16,18)-W-[FYH]-H-x(2)-C [The three C's and the H are zinc ligands] Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROT NONE. Sequences known to belong to this class detected by the profile ALL. Other sequence(s) detected in SWISS-PROT NONE.

Note: This documentation entry is linked to both signature patterns and a profile. As the profile is much more sensitive than the patterns, you should use it if you have access to the necessary software tools to do so.

[1] Althaus F.R., Richter C.R. Mol. Biol. Biochem. Biophys. 37:1-126(1987).
 [2] de Murcia G., Menissier de Murcia J. Trends Biochem. Sci. 19:172-176(1994).
 [3] Wei Y.-F., Robins P., Carter K., Caldecott K., Pappin D.J.C., Yu G.-L., Wang R.-P., Shell B.K., Nash R.A., Schar P., Barnes D.E., Haseltine W.A., Lindahl T. Mol. Cell. Biol. 15:3206-3216(1995).

882. Adenylylsulfate kinase (APS_kinase)

Enzyme that catalyses the phosphorylation of adenylylsulfate to 3'-phosphoadenylylsulfate. This domain contains an ATP binding P-loop motif. Number of members: 34

[1] MacRae IJ, Rose AB, Segel IH; Medline: 99003196 Adenosine 5'-phosphosulfate kinase from *Penicillium chrysogenum*. site- directed mutagenesis at putative phosphoryl-accepting and ATP P-loop residues. J Biol Chem 1998;273:28583-28589.

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883. DNA polymerase family B signature DNA_POLYMERASE_B (DNA_pol_B)

Replicative DNA polymerases (EC 2.7.7.7) are the key enzymes catalyzing the accurate replication of DNA. They require either a small RNA molecule or a protein as a primer for the de novo synthesis of a DNA chain. On the basis of sequence similarity, a number of DNA polymerases have been grouped [1 to 7] under the designation of DNA polymerase family B. These are:

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- Higher eukaryotes polymerases alpha.
- Higher eukaryotes polymerases delta.
- Yeast polymerase I/alpha (gene POL1), polymerase II/epsilon (gene POL2), polymerase III/delta (gene POL3) and polymerase REV3.
- *Escherichia coli* polymerase II (gene *dinA* or *polB*).
- Archaeobacterial polymerases.
- Polymerases of viruses from the herpesviridae family.
- Polymerases from Adenoviruses.
- Polymerases from Baculoviruses.
- Polymerases from Chlorella viruses.
- Polymerases from Poxviruses.
- Bacteriophage T4 polymerase.
- Podoviridae bacteriophages Phi-29, M2 and PZA polymerase.

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- Tectiviridae bacteriophage PRD1 polymerase.

- Polymerases encoded on mitochondrial linear DNA plasmids in various fungi and plants (*Kluyveromyces lactis* pGKL1 and pGKL2, *Agaricus bitorquis* pEM, *Ascobolus immersus* pAI2, *Claviceps purpurea* pCLK1, *Neurospora Kalilo* and Maranhar, maize S-1, etc).

Six regions of similarity (numbered from I to VI) are found in all or a subset of the above polymerases. The most conserved region (I) includes a conserved tetrapeptide with two aspartate residues. Its function is not yet known. However, it has been suggested [3] that it may be involved in binding a magnesium ion. This conserved region was selected as a signature for this family of DNA polymerases.

Consensus pattern [YAJ]-[GLIVMSTAC]-D-T-D-[SG]-[LIVMFTC]-x-[LIVMSTAC]
Sequences known to belong to this class detected by the patternALL, except for yeast
polymerase II/epsilon, Agaricus bitorquis pEM and Sulfolobus solfataricus polymerase II.

- [1] Jung G., Leavitt M.C., Hsieh J.-C., Ito J. Proc. Natl. Acad. Sci. U.S.A. 84:8287-8291(1987).
- [2] Bernad A., Zaballos A., Salas M., Blanco L. EMBO J. 6:4219-4225(1987).
- [3] Argos P. Nucleic Acids Res. 16:9909-9916(1988).
- [4] Wang T.S.-F., Wong S.W., Korn D. FASEB J. 3:14-21(1989).
- [5] Delarue M., Poch O., Todro N., Moras D., Argos P. Protein Eng. 3:461-467(1990).
- [6] Ito J., Braithwaite D.K. Nucleic Acids Res. 19:4045-4057(1991).
- [7] Braithwaite D.K., Ito J. Nucleic Acids Res. 21:787-802(1993).

884. DNA polymerase family X signature - DNA_POLYMERASE_X (DNA_polymeraseX)

DNA polymerases (EC 2.7.7.7) can be classified, on the basis of sequence similarity [1], into
at least four different groups: A, B, C and X. DNA polymerases that belong to family X are
listed below [2]:

- Vertebrate polymerase beta, involved in DNA repair.
- Yeast polymerase IV (POL4) [3], an enzyme with similar characteristics to that of the
mammalian polymerase beta.
- Terminal deoxynucleotidyltransferase (TdT) (EC 2.7.7.31). TdT catalyzes the elongation of
polydeoxynucleotide chains by terminal addition. One of the functions of this enzyme is the
addition of nucleotides at the junction of rearranged Ig heavy chain and T cell receptor gene
segments during the maturation of B and T cells.
- African Swine Fever virus protein O174L [4].
- Fission yeast hypothetical protein SpAC2F7.06c.

These enzymes are small (about 40 Kd) compared with other polymerases and their reaction
mechanism operates via a distributive mode, i.e. they dissociate from the template-primer
after addition of each nucleotide.

As a signature pattern for this family of DNA polymerases, a highly conserved region that contains a conserved arginine and two conserved aspartic acid residues were selected. The latter together with the arginine have been shown [5] to be involved in primer binding in polymerase beta.

Consensus pattern G-[SG]-[LFY]-x-R-[GE]-x(3)-[SGCL]-x-D-[LIVM]-D-[LIVMFY](3)-x(2)-[SAP] Sequences known to belong to this class detected by the patternALL.

[1] Ito J., Braithwaite D.K. Nucleic Acids Res. 19:4045-4057(1991).

[2] Matsukage A., Nishikawa K., Ooi T., Seto Y., Yamaguchi M. J. Biol. Chem. 262:8960-8962(1987).

[3] Prasad R., Widen S.G., Singhal R.K., Watkins J., Prakash L., Wilson S.H. Nucleic Acids Res. 21:5301-5307(1993).

[4] Yanez R.J., Rodriguez J.M., Nogal M.L., Yuste L., Enriquez C., Rodriguez J.F., Vinuela E. Virology 208:249-278(1995).

[5] Date T., Yamamoto S., Tanihara K., Nishimoto Y., Matsukage A. Biochemistry 30:5286-5292(1991).

885. DUF14 - Domain of unknown function

This domain is found in glutamate synthase, tungsten formylmethanofuran dehydrogenase subunit c (FwdC) and molybdenum formylmethanofuran dehydrogenase subunit c (FmdC). It has no known function. Number of members: 52

[1] Hochheimer A, Hedderich R, Thauer RK; Medline: 99035764. The formylmethanofuran dehydrogenase isoenzymes in Methanobacterium wolfei and Methanobacterium thermoautotrophicum: induction of the molybdenum isoenzyme by molybdate and constitutive synthesis of the tungsten isoenzyme." Arch Microbiol 1998;170:389-393.

886. DUF18-Domain of unknown function

This domain of unknown function is found in several C. elegans proteins. The domain is 120 amino acids long and rich in cysteine residues. There are 16 conserved cysteine positions in the domain. Number of members: 34

887. DUF27-Domain of unknown function

This domain is found in a number of otherwise unrelated proteins. This domain is found at the C-terminus of the macro-H2A histone protein Swiss:Q02874. This domain is found in the non-structural proteins of several types of ssRNA viruses such as NSP2 from alphaviruses Swiss:P03317. This domain is also found on its own in a family of proteins from bacteria Swiss:P75918, archaeobacteria Swiss:O59182 and eukaryotes Swiss:Q17432, suggesting that it is involved in an important and ubiquitous cellular process. Number of members: 66

888. DUF37-Domain of unknown function

This domain is found in short (70 amino acid) hypothetical proteins from various bacteria.

The domain contains three conserved cysteine residues. Swiss:Q44066 from *Aeromonas hydrophila* has been found to have hemolytic activity (unpublished). Number of members: 19

889. EGF-like domain signatures. (EGF-like)

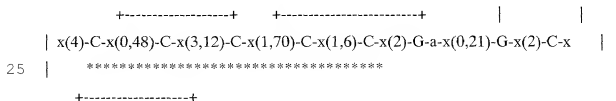
A sequence of about thirty to forty amino-acid residues long found in the sequence of epidermal growth factor (EGF) has been shown [1 to 6] to be present, in a more or less conserved form, in a large number of other, mostly animal proteins. The proteins currently known to contain one or more copies of an EGF-like pattern are listed below.

- Adipocyte differentiation inhibitor (gene PREF-1) from mouse (6 copies).
- Agrin, a basal lamina protein that causes the aggregation of acetylcholine receptors on cultured muscle fibers (4 copies).
- Amphiregulin, a growth factor (1 copy).
- Betacellulin, a growth factor (1 copy).
- Blastula proteins BP10 and Span from sea urchin which are thought to be involved in pattern formation (1 copy).
- BM86, a glycoprotein antigen of cattle tick (7 copies).
- Bone morphogenic protein 1 (BMP-1), a protein which induces cartilage and bone formation and which expresses metalloendopeptidase activity (1-2 copies). Homologous proteins are found in sea urchin - suBMP (1 copy) - and in *Drosophila* - the dorsal-ventral patterning protein tolloid (2 copies).
- *Caenorhabditis elegans* developmental proteins lin-12 (13 copies) and glp-1 (10 copies).
- *Caenorhabditis elegans* APX-1 protein, a patterning protein (4.5 copies).
- Calcium-dependent serine proteinase (CASP) which degrades the extracellular matrix proteins type I and IV collagen and fibronectin (1 copy).

- Cartilage matrix protein CMP (1 copy).
- Cartilage oligomeric matrix protein COMP (4 copies).
- Cell surface antigen 114/A10 (3 copies).
- Cell surface glycoprotein complex transmembrane subunit ASGP-2 from rat (2 copies).
- 5 - Coagulation associated proteins C, Z (2 copies) and S (4 copies).
- Coagulation factors VII, IX, X and XII (2 copies).
- Complement C1r components (1 copy).
- Complement C1s components (1 copy).
- Complement-activating component of Ra-reactive factor (RARF) (1 copy).
- 10 - Complement components C6, C7, C8 alpha and beta chains, and C9 (1 copy).
- Crumbs, an epithelial development protein from Drosophila (29 copies).
- Epidermal growth factor precursor (7-9 copies).
- Exogastrula-inducing peptides A, C, D and X from sea urchin (1 copy).
- Fat protein, a Drosophila cadherin-related tumor suppressor (5 copies).
- 15 - Fetal antigen 1, a probable neuroendocrine differentiation protein, which is derived from the delta-like protein (DLK) (6 copies).
- Fibrillin 1 (47 copies) and fibrillin 2 (14 copies).
- Fibropellins IA (21 copies), IB (13 copies), IC (8 copies), II (4 copies) and III (8 copies) from the apical lamina - a component of the extracellular matrix - of sea urchin.
- 20 - Fibulin-1 and -2, two extracellular matrix proteins (9-11 copies).
- Giant-lens protein (protein Argos), which regulates cell determination and axon guidance in the Drosophila eye (1 copy).
- Growth factor-related proteins from various poxviruses (1 copy).
- Gurken protein, a Drosophila developmental protein (1 copy).
- 25 - Heparin-binding EGF-like growth factor (HB-EGF), transforming growth factor alpha (TGF-alpha), growth factors Lin-3 and Spitz (1 copy); the precursors are membrane proteins, the mature form is located extracellular.
- Hepatocyte growth factor (HGF) activator (EC 3.4.21.-) (2 copies).
- LDL and VLDL receptors, which bind and transport low-density lipoproteins and very low-density lipoproteins (3 copies).
- 30 - LDL receptor-related protein (LRP), which may act as a receptor for endocytosis of extracellular ligands (22 copies).
- Leucocyte antigen CD97 (3 copies), cell surface glycoprotein EMR1 (6 copies) and cell surface glycoprotein F4/80 (7 copies).

- Limulus clotting factor C, which is involved in hemostasis and host defense mechanisms in Japanese horseshoe crab (1 copy).
 - Mceprn A alpha subunit, a mammalian membrane-bound endopeptidase (1 copy).
 - Milk fat globule-EGF factor 8 (MFG-E8) from mouse (2 copies).
- 5 - Neuregulin GGF-I and GGF-II, two human glial growth factors (1 copy).
- Neurexins from mammals (3 copies).
 - Neurogenic proteins Notch, Xotch and the human homolog Tan-1 (36 copies), Delta (9 copies) and the similar differentiation proteins Lag-2 from *Caenorhabditis elegans* (2 copies), Serrate (14 copies) and Slit (7 copies) from *Drosophila*.
- 10 - Nidogen (also called entactin), a basement membrane protein from chordates (2-6 copies).
- Ookinete surface proteins (24 Kd, 25 Kd, 28 Kd) from *Plasmodium* (4 copies).
 - Pancreatic secretory granule membrane major glycoprotein GP2 (1 copy).
 - Perforin, which lyses non-specifically a variety of target cells (1 copy).
 - Proteoglycans aggrecan (1 copy), versican (2 copies), perlecan (at least 2 copies), brevican (1 copy) and chondroitin sulfate proteoglycan (gene PG-M) (2 copies).
- 15 - Prostaglandin G/H synthase 1 and 2 (EC 1.14.99.1) (1 copy), which is found in the endoplasmic reticulum.
- S1-5, a human extracellular protein whose ultimate activity is probably modulated by the environment (5 copies).
- 20 - Schwannoma-derived growth factor (SDGF), an autocrine growth factor as well as a mitogen for different target cells (1 copy).
- Selectins. Cell adhesion proteins such as ELAM-1 (E-selectin), GMP-140 (P-selectin), or the lymph-node homing receptor (L-selectin) (1 copy).
 - Serine/threonine-protein kinase homolog (gene Pro25) from *Arabidopsis thaliana*, which may be involved in assembly or regulation of light-harvesting chlorophyll A/B protein (2 copies).
- 25 - Sperm-egg fusion proteins PH-30 alpha and beta from guinea pig (1 copy).
- Stromal cell derived protein-1 (SCP-1) from mouse (6 copies).
 - TDGF-1, human teratocarcinoma-derived growth factor 1 (1 copy).
- 30 - Tenascin (or neuronectin), an extracellular matrix protein from mammals (14.5 copies), chicken (TEN-A) (13.5 copies) and the related proteins human tenascin-X (18 copies) and tenascin-like proteins TEN-A and TEN-M from *Drosophila* (8 copies).
- Thrombomodulin (fetomodulin), which together with thrombin activates protein C (6 copies).

- Thrombospondin 1, 2 (3 copies), 3 and 4 (4 copies), adhesive glycoproteins that mediate cell-to-cell and cell-to-matrix interactions.
- Thyroid peroxidase 1 and 2 (EC 1.11.1.8) from human (1 copy).
- Transforming growth factor beta-1 binding protein (TGF-B1-BP) (16 or 18 copies).
- 5 - Tyrosine-protein kinase receptors Tek and Tie (EC 2.7.1.112) (3 copies).
- Urokinase-type plasminogen activator (EC 3.4.21.73) (UPA) and tissue plasminogen activator (EC 3.4.21.68) (TPA) (1 copy).
- Uromodulin (Tamm-horsfall urinary glycoprotein) (THP) (3 copies).
- Vitamin K-dependent anticoagulants protein C (2 copies) and protein S (4 copies) and the
- 10 similar protein Z, a single-chain plasma glycoprotein of unknown function (2 copies).
- 63 Kd sperm flagellar membrane protein from sea urchin (3 copies).
- 93 Kd protein (gene nel) from chicken (5 copies).
- Hypothetical 337.6 Kd protein T20G5.3 from *Caenorhabditis elegans* (44 copies).
- 15 The functional significance of EGF domains in what appear to be unrelated proteins is not yet clear. However, a common feature is that these repeats are found in the extracellular domain of membrane-bound proteins or in proteins known to be secreted (exception: prostaglandin G/H synthase). The EGF domain includes six cysteine residues which have been shown (in EGF) to be involved in disulfide bonds. The main structure is a two-stranded beta-sheet
- 20 followed by a loop to a C-terminal short two-stranded sheet. Subdomains between the conserved cysteines strongly vary in length as shown in the following schematic representation of the EGF-like domain:



'C': conserved cysteine involved in a disulfide bond.

'G': often conserved glycine

30 'a': often conserved aromatic amino acid

'*': position of both patterns.

'x': any residue

The region between the 5th and 6th cysteine contains two conserved glycines of which at least one is present in most EGF-like domains. Two patterns were created for this domain, each including one of these C-terminal conserved glycine residues.

- 5 Consensus pattern: C-x-C-x(5)-G-x(2)-C [The 3 C's are involved in disulfide bonds]
Sequences known to belong to this class detected by the pattern A majority, but not those that have very long or very short regions between the last 3 conserved cysteines of their EGF-like domain(s). Other sequence(s) detected in SWISS-PROT87 proteins, of which 27 can be considered as possible candidates.

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Consensus pattern: C-x-C-x(2)-[GP]-[FYW]-x(4,8)-C [The three C's are involved in disulfide bonds] Sequences known to belong to this class detected by the pattern A majority, but not those that have very long or very short regions between the last 3 conserved cysteines of their EGF-like domain(s). Other sequence(s) detected in SWISS-PROT83 proteins, of which 49
15 can be considered as possible candidates. Note The beta chain of the integrin family of proteins contains 2 cysteine- rich repeats which were said to be dissimilar with the EGF pattern [7].

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Note Laminin EGF-like repeats (see <PDOC00961>) are longer than the average EGF module and contain a further disulfide bond C-terminal of the EGF-like region. Perlecan and agrin contain both EGF-like domains and laminin-type EGF-like domains. Note the pattern do not detect all of the repeats of proteins with multiple EGF-like repeats. Note see <PDOC00913> for an entry describing specifically the subset of EGF- like domains that bind calcium.

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[1] Davis C.G. New Biol. 2:410-419(1990).

[2] Blomquist M.C., Hunt L.T., Barker W.C. Proc. Natl. Acad. Sci. U.S.A. 81:7363-7367(1984).

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[3] Barker W.C., Johnson G.C., Hunt L.T., George D.G. Protein Nucl. Acid Enz. 29:54-68(1986).

[4] Doolittle R.F., Feng D.F., Johnson M.S. Nature 307:558-560(1984).

[5] Appella E., Weber I.T., Blasi F. FEBS Lett. 231:1-4(1988).

[6] Campbell I.D., Bork P. Curr. Opin. Struct. Biol. 3:385-392(1993).

[7] Tamkun J.W., DeSimone D.W., Fonda D., Patel R.S., Buck C., Horwitz A.F., Hynes R.O. Cell 46:271-282(1986).

5 890. Ham1 family (Ham1p_like)

This family consists of the HAM1 protein Swiss:P47119 and hypothetical archaeal bacterial and C. elegans proteins. HAM1 controls 6-N-hydroxylaminopurine (HAP) sensitivity and mutagenesis in S. cerevisiae Swiss:P47119 [1]. The HAM1 protein protects the cell from HAP, either on the level of deoxynucleoside triphosphate or the DNA level by a yet unidentified set of reactions [1]. Number of members: 19

[1] Noskov VN, Staak K, Shcherbakova PV, Kozmin SG, Negishi K, Ono BC, Hayatsu H, Pavlov YI; Medline: 96381244 HAM1, the gene controlling 6-N-hydroxylaminopurine sensitivity and mutagenesis in the yeast Saccharomyces cerevisiae." Yeast 1996;12:17-29.

15 891. (HCO3_cotransp)

Anion exchange is a cellular transport function which contributes to the regulation of cell pH and volume. Anion exchangers are a family of functionally related proteins that contributes to these properties by maintaining the intracellular level of the two principal anions: chloride and HCO₃⁻. The best characterized anion exchanger is the band 3 protein [1], which is an erythrocyte anion exchange membrane glycoprotein. Band 3 is a protein of about 900 amino acids which consists of a cytoplasmic N-terminal domain of about 400 residues and an hydrophobic C-terminal section of about 500 residues that contains at least ten transmembrane regions. The cytoplasmic domain provides binding sites for cytoskeletal proteins, while the integral membrane domain is responsible for anion transport. Band 3 protein is specific to erythroid cells, at least two other proteins [2] structurally and functionally related to band 3, are found in nonerythroid tissues:

25 - AE2 (or B3 related protein; B3RP), a protein of 1200 residues, which seems to be present in a variety of cell types including lymphoid, kidney, and choroid plexus.

30 - AE3, a protein of 1200 residues, which is specific to neurons.

Structurally AE2 and AE3 are very similar to band 3, the main difference being an extension of some 300 residues of the N-terminal domain in AE2 and AE3.

Two signature patterns were developed for these proteins. The first pattern is based on a conserved stretch of sequence that contains four clustered positive charged residues and which is located at the C-terminal extremity of the cytoplasmic domain, just before the first transmembrane segment from the integral domain. The second pattern is based on the perfectly conserved sequence of the fifth transmembrane segment; this segment contains a lysine, which is the covalent binding site for the isothiocyanate group of DIDS, an inhibitor of anion exchange.

Consensus pattern F-G-G-[LIVM](2)-[KR]-D-[LIVM]-[RK]-R-R-Y Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [FI]-L-I-S-L-I-F-I-Y-E-T-F-x-K-L Sequences known to belong to this class detected by the pattern ALL.

- [1] Jay D., Cantley L. Annu. Rev. Biochem. 55:511-538(1986).
- [2] Reithmeier R.A.F. Curr. Opin. Struct. Biol. 3:515-523(1993).

892. ATP phosphoribosyltransferase signature (HisG)

ATP phosphoribosyltransferase (EC 2.4.2.17) is the enzyme that catalyzes the first step in the biosynthesis of histidine in bacteria, fungi and plants. It is a protein of about 23 to 32 Kd. As a signature pattern a region located in the C-terminal part of this enzyme was selected.

Consensus pattern E-x(5)-G-x-[SAG]-x(2)-[IV]-x-D-[LIV]-x(2)-[ST]-G-x-T-[LM] Sequences known to belong to this class detected by the pattern ALL.

893. HNH endonuclease (HNH)

Number of members: 56

- [1] Shub DA, Goodrich-Blair H, Eddy SR; Medline: 95117127 Amino acid sequence motif of group I intron endonucleases is conserved in open reading frames of group II introns." Trends Biochem Sci 1994;19:402-404.
- [2] Dalgaard JZ, Klar AJ, Moser MJ, Holley WR, Chatterjee A, Mian IS; Medline: 98026854 Statistical modeling and analysis of the LAGLIDADG family of site- specific endonucleases

and identification of an intein that encodes a site-specific endonuclease of the HNH family." *Nucleic Acids Res* 1997;25:4626-4638.

[3] Gorbalenya AE; Medline: 95004046 Self-splicing group I and group II introns encode homologous (putative) DNA endonucleases of a new family." *Protein Sci* 1994;3:1117-1120.

894. NEUROHYPOPHYS_HORM (hormone5)

Oxytocin (or ocytocin) and vasopressin [1] are small (nine amino acid residues), structurally and functionally related neurohypophysial peptide hormones. Oxytocin causes contraction of the smooth muscle of the uterus and of the mammary gland while vasopressin has a direct antidiuretic action on the kidney and also causes vasoconstriction of the peripheral vessels.

Like the majority of active peptides, both hormones are synthesized as larger protein precursors that are enzymatically converted to their mature forms. Peptides belonging to this family are also found in birds, fish, reptiles and amphibians (mesotocin, isotocin, valitocin, glutitocin, aspartocin, vasotocin, seritocin, asvatocin, phasvatocin), in worms (annetocin), octopi (cephalotocin), locust (locupressin or neuropeptide F1/F2) and in molluscs (conopressins G and S) [2]. The pattern developed to detect this category of peptides spans their entire sequence and includes four invariant amino acid residues.

Consensus pattern C-[LIFY](2)-x-N-[CS]-P-x-G [The two C's are linked by a disulfide bond]. Sequences known to belong to this class detected by the pattern ALL.

[1] Acher R., Chauvet J. *Biochimie* 70:1197-1207(1988).

[2] Chauvet J., Michel G., Ouedraogo Y., Chou J., Chait B.T., Acher R. *Int. J. Pept. Protein Res.* 45:482-487(1995).

895. 7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase (HPPK)

All organisms require reduced folate cofactors for the synthesis of a variety of metabolites. Most microorganisms must synthesize folate de novo because they lack the active transport system of higher vertebrate cells which allows these organisms to use dietary folates. Enzymes involved in folate biosynthesis are therefore targets for a variety of antimicrobial agents such as trimethoprim or sulfonamides. 7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase (EC 2.7.6.3) (HPPK) catalyzes the attachment of pyrophosphate to 6-hydroxymethyl-7,8-dihydropterin to form 6-hydroxymethyl-7,8-dihydropteridine

pyrophosphate. This is the first step in a three-step pathway leading to 7,8-dihydrofolate. Bacterial HPPK (gene folK or sulD) [1] is a protein of 160 to 270 amino acids. In the lower eukaryote *Pneumocystis carinii*, HPPK is the central domain of a multifunctional folate synthesis enzyme (gene fas) [2]. As a signature for HPPK, a conserved region located in the central section of these enzymes was selected.

Consensus pattern [KRHD]-x-[GA]-[PSAE]-R-x(2)-D-[LIV]-D-[LIVM](2) Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROT/NONE.

- [1] Talarico T.L., Ray P.H., Dev I.K., Merrill B.M., Dallas W.S. J. Bacteriol. 174:5971-5977(1992).
 [2] Volpes F., Dyer M., Scaife J.G., Darby G., Stammers D.K., Delves C.J. Gene 112:213-218(1992).

896. Metalloenzyme superfamily (Metalloenzyme)

This family includes phosphopentomutase Swiss:P07651 and 2,3-bisphosphoglycerate-independent phosphoglycerate mutase, Swiss:P37689. This family is also related to alk_phosphatase [1]. The alignment contains the most conserved residues that are probably involved in metal binding and catalysis. Number of members: 34

- [1] Galperin MY, Bairoch A, Koonin EV; Medline: 99180418 A superfamily of metalloenzymes unifies phosphopentomutase and cofactor- independent phosphoglycerate mutase with alkaline phosphatases and sulfatases." Protein Sci 1998;7:1829-1835.

897. Penicillin amidase (Penicil_amidase)

Penicillin amidase or penicillin acylase EC:3.5.1.11 catalyses the hydrolysis of benzylpenicillin to phenylacetic acid and 6-aminopenicillanic acid (6-APA) a key intermediate in the synthesis of penicillins [1]. Also in the family is cephalosporin acylase Swiss:P07662 and Swiss:P29958 aculeacin A acylase which are involved in the synthesis of related peptide antibiotics. Number of members: 13

[1] Verhaert RM, Riemens AM, van der Laan JM, van Duin J, Quax WJ; Medline: 97438505
Molecular cloning and analysis of the gene encoding the thermostable penicillin G acylase
from *Alcaligenes faecalis*. *Appl Environ Microbiol* 1997;63:3412-3418.

[2] Duggleby HJ, Tolley SP, Hill CP, Dodson EJ, Dodson G, Moody PC; Medline: 95115804
5 Penicillin acylase has a single-amino-acid catalytic centre." *Nature* 1995;373:264-268.

898. Phosphoribosyl-AMP cyclohydrolase (PRA-CH)

This enzyme catalyses the third step in the histidine biosynthetic pathway. It requires Zn ions
10 for activity. Number of members: 13

[1] D'Ordine RL, Klem TJ, Davisson VJ; Medline: 99129952 N1-(5'-
phosphoribosyl)adenosine-5'-monophosphate cyclohydrolase: purification and
characterization of a unique metalloenzyme. *Biochemistry* 1999;38:1537-1546.

899. Phosphoribosyl-ATP pyrophosphohydrolase (PRA-PH)

This enzyme catalyses the second step in the histidine biosynthetic pathway. Number of
members: 32

[1] Keesey JK Jr, Bigelis R, Fink GR; Medline: 79216449 The product of the *his4* gene
cluster in *Saccharomyces cerevisiae*. A trifunctional polypeptide." *J Biol Chem* 1979 Aug
10;254:7427-7433.

[2] Bruni CB, Carlomagno MS, Formisano S, Paoletta G; Medline: 86310274 Primary and
25 secondary structural homologies between the *HIS4* gene product of *Saccharomyces*
cerevisiae and the *hisIE* and *hisD* gene products of *Escherichia coli* and *Salmonella*
typhimurium." *Mol Gen Genet* 1986;203:389-396.

900. Prokaryotic membrane lipoprotein lipid attachment site (PstS)

In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which
is cleaved by a specific lipoprotein signal peptidase (signal peptidase II). The peptidase
recognizes a conserved sequence and cuts upstream of a cysteine residue to which a

glyceride-fatty acid lipid is attached [1]. Some of the proteins known to undergo such processing currently include (for recent listings see [1,2,3]):

- Major outer membrane lipoprotein (murein-lipoproteins) (gene *lpp*).
- *Escherichia coli* lipoprotein-28 (gene *nlpA*).
- 5 - *Escherichia coli* lipoprotein-34 (gene *nlpB*).
- *Escherichia coli* lipoprotein *nlpC*.
- *Escherichia coli* lipoprotein *nlpD*.
- *Escherichia coli* osmotically inducible lipoprotein B (gene *osmB*).
- *Escherichia coli* osmotically inducible lipoprotein E (gene *osmE*).
- 10 - *Escherichia coli* peptidoglycan-associated lipoprotein (gene *pal*).
- *Escherichia coli* rare lipoproteins A and B (genes *rplA* and *rplB*).
- *Escherichia coli* copper homeostasis protein *cutF* (or *nlpE*).
- *Escherichia coli* plasmids *traT* proteins.
- *Escherichia coli* Col plasmids lysis proteins.
- 15 - A number of *Bacillus* beta-lactamases.
- *Bacillus subtilis* periplasmic oligopeptide-binding protein (gene *oppA*).
- *Borrelia burgdorferi* outer surface proteins A and B (genes *ospA* and *ospB*).
- *Borrelia hermsii* variable major protein 21 (gene *vmp21*) and 7 (gene *vmp7*).
- *Chlamydia trachomatis* outer membrane protein 3 (gene *omp3*).
- 20 - *Fibrobacter succinogenes* endoglucanase *cel-3*.
- *Haemophilus influenzae* proteins *Pal* and *Pcp*.
- *Klebsiella* pullulanase (gene *pulA*).
- *Klebsiella* pullulanase secretion protein *pulS*.
- *Mycoplasma hyorhinis* protein *p37*.
- 25 - *Mycoplasma hyorhinis* variant surface antigens A, B, and C (genes *vlpABC*).
- *Neisseria* outer membrane protein *H.8*.
- *Pseudomonas aeruginosa* lipopeptide (gene *lppL*).
- *Pseudomonas solanacearum* endoglucanase *egl*.
- *Rhodospseudomonas viridis* reaction center cytochrome subunit (gene *cytC*).
- 30 - *Rickettsia* 17 Kd antigen.
- *Shigella flexneri* invasion plasmid proteins *mxlJ* and *mxlM*.
- *Streptococcus pneumoniae* oligopeptide transport protein A (gene *amiA*).
- *Treponema pallidum* 34 Kd antigen.
- *Treponema pallidum* membrane protein A (gene *tmpA*).

- *Vibrio harveyi* chitobiase (gene chb).
- *Yersinia* virulence plasmid protein yscJ.
- Halocyanin from *Natrobacterium pharaonis* [4], a membrane associated copper-binding protein. This is the first archaeobacterial protein known to be modified in such a fashion).

5 From the precursor sequences of all these proteins, a consensus pattern was derived and a set of rules to identify this type of post-translational modification.

Consensus pattern {DERK}(6)-[LIVFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1) The cysteine must be between positions 15 and 35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the seven positions of the sequence. Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROT some 100 prokaryotic proteins. Some of them are not membrane lipoproteins, but at least half of them could be.

- 15 [1] Hayashi S., Wu H.C. J. Bioenerg. Biomembr. 22:451-471(1990).
 [2] Klein P., Somorjai R.L., Lau P.C.K. Protein Eng. 2:15-20(1988).
 [3] von Heijne G. Protein Eng. 2:531-534(1989).
 [4] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

901. Ribosome recycling factor (RRF)

The ribosome recycling factor (RRF / ribosome release factor) dissociates the ribosome from the mRNA after termination of translation, and is essential bacterial growth [1]. Thus
 25 ribosomes are "recycled" and ready for another round of protein synthesis. Number of members: 27

[1] Janosi L, Shimizu I, Kaji A; Medline: 94240115 Ribosome recycling factor (ribosome releasing factor) is essential for bacterial growth." Proc Natl Acad Sci U S A 1994;91:4249-
 30 4253.

902. S-layer homology (SLH)

S-layers are paracrystalline mono-layered assemblies of (glyco)proteins which coat the surface of bacteria [1]. Several S-layer proteins and some other cell wall proteins contain one or more copies of a domain of about 50-60 residues, which has been called SLH (for S-layer homology) [2]. There is strong evidence that this domain serves as an anchor to the

- 5 peptidoglycan [3]. The SLH domain has been found in:
 - S-layer glycoprotein of *Acetogenium kivui* (3 copies).
 - S-layer 125 Kd protein of *Bacillus sphaericus* (3 copies).
 - S-layer protein of *Bacillus anthracis* (3 copies).
 - S-layer protein of *Bacillus licheniformis* (3 copies).
- 10
 - S-layer protein (HWP) from *Bacillus brevis* strain HPD31 (3 copies).
 - Middle cell wall protein (MWP) from *Bacillus brevis* strain 47 (3 copies).
 - S-layer protein (p100) of *Thermus thermophilus* (1 copy).
 - Outer membrane protein Omp-alpha from *Thermotoga maritima* (1 copy).
 - Cellulosome anchoring protein (gene *ancA*), outer layer protein B (OlpB) and a further
- 15 potential cell surface glycoprotein from *Clostridium thermocellum* (3 copies; the first copy is missing its N-terminal third which is appended to the end of the third copy; may have arisen by circular permutation).
 - Amylopullulanase (gene *amyB*) from *Thermoanaerobacter thermosulfurogenes* (3 copies)
 - Amylopullulanase (gene *aapT*) from *Bacillus* strain XAL-601 (3 copies).
- 20
 - Endoglucanase from *Bacillus* strain KSM-635 (3 copies).
 - Exoglucanase (gene *xynX*) from *Clostridium thermocellum* (3 copies).
 - Xylanase A (gene *xynA*) from *Thermoanaerobacter saccharolyticum* (2 copies; 3 copies if a frameshift is taken into account).
 - Protein involved in butirosin production (ButB) from *Bacillus circulans* (2 incomplete
- 25 copies; 3 copies if three frameshifts are taken into account).
 - Two hypothetical proteins from *Synechocystis* strain PCC 6803 (1 copy each).
 - A hypothetical protein with sequence similarity to amylopullulanases found 3' of amylase gene from *Bacillus circulans* (fragment of 1 copy; 3 copies if two frameshifts are taken into account).
- 30 SLH domains are found at the N- or C-termini of mature proteins. They occur in single copy followed by a predicted coiled coil domain, or in three contiguous copies. Structurally, the SLH domain is predicted to contain two alpha-helices flanking a beta strand. The SLH sequences are fairly divergent with an average identity of about 25%. It is however possible

to build a sequence pattern that starts at the second position of the domain and that spans 3/4 of its length.

Consensus pattern[LVFYT]-x-[DA]-x(2,5)-[DNGSATPHY]-[FYWPD A]-x(4)-[LIV]-x(2)-
 5 [GTALV]-x(4,6)-[LIVFYC]-x(2)-G-x-[PGSTA]-x(2,3)-[MFYA]-x- [PGAV]-x(3,10)-
 [LIVMA]-[STKR]-[RY]-x-[EQ]-x-[STALIVM] Sequences known to belong to this class
 detected by the pattern ALL. Other sequence(s) detected in SWISS-PROT NONE.

[1] Beveridge T.J. Curr. Opin. Struct. Biol. 4:204-212(1994).

10 [2] Lupas A., Engelhardt H., Peters J., Santarius U., Volker S., Baumeister W. J. Bacteriol.
 176:1224-1233(1994).

[3] Lemaire M., Ohayon H., Gounon P., Fujino T., Beguin P. J. Bacteriol. 177:2451-
 2459(1995).

903. Queuine tRNA-ribosyltransferase (TGT)

This is a family of queuine tRNA-ribosyltransferases EC:2.4.2.29, also known as tRNA-
 guanine transglycosylase and guanine insertion enzyme. Queuine tRNA-ribosyltransferase
 modifies tRNAs for asparagine, aspartic acid, histidine and tyrosine with queuine. It catalyses
 20 the exchange of guanine-34 at the wobble position with 7-aminomethyl-7-deazaguanine, and
 the addition of a cyclopentenediol moiety to 7-aminomethyl-7-deazaguanine-34 tRNA;
 giving a hypermodified base queuine in the wobble position [1,2]. The aligned region contains
 a zinc binding motif C-x-C-x2-C-x29-H, and important tRNA and 7-aminomethyl-
 7-deazaguanine binding residues [1]. Number of members: 27

[1] Romier C, Reuter K, Suck D, Ficner R; Medline: 96256303 Crystal structure of tRNA-
 guanine transglycosylase: RNA modification by base exchange." EMBO J 1996;15:2850-
 2857.

[2] Garcia GA, Koch KA, Chong S; Medline: 93287116 tRNA-guanine transglycosylase
 30 from Escherichia coli. Overexpression, purification and quaternary structure." J Mol Biol
 1993;231:489-497.

904. ThiC Family (ThiC)

ThiC is found within the thiamine biosynthesis operon. ThiC is involved in pyrimidine biosynthesis [2]. ThiC catalyzes the substitution of the pyrophosphate of 2-methyl-4-amino-5-hydroxymethylpyrimidine pyrophosphate by 4-methyl-5-(beta-hydroxyethyl)thiazole phosphate to yield thiamine phosphate [3]. Number of members: 12

5

[1] Vander Horn PB, Backstrom AD, Stewart V, Begley TP; Medline: 93163063 Structural genes for thiamine biosynthetic enzymes (thiCEFGH) in *Escherichia coli* K-12." *J Bacteriol* 1993;175:982-992.

[2] Begley TP, Downs DM, Ealick SE, McLafferty FW, Van Loon AP, Taylor S,

10

Campobasso N, Chiu HJ, Kinsland C, Reddick JJ, Xi J; Medline: 99311269 Thiamin biosynthesis in prokaryotes." *Arch Microbiol* 1999;171:293-300.

[3] Zhang Y, Taylor SV, Chiu HJ, Begley TP; Medline: 97284509 Characterization of the *Bacillus subtilis* thiC operon involved in thiamine biosynthesis." *J Bacteriol* 1997;179:3030-3035.

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905. Putative tRNA binding domain (tRNA_bind)

This domain is found in prokaryotic methionyl-tRNA synthetases, prokaryotic phenylalanyl tRNA synthetases the yeast GU4 nucleic-binding protein (G4p1 or p42, ARC1) [2], human tyrosyl-tRNA synthetase [1], and endothelial-monocyte activating polypeptide II. G4p1 binds specifically to tRNA form a complex with methionyl-tRNA synthetases [2]. In human tyrosyl-tRNA synthetase this domain may direct tRNA to the active site of the enzyme [2]. This domain may perform a common function in tRNA aminoacylation [1]. Number of members: 12

25

[1] Kleeman TA, Wei D, Simpson KL, First EA; Medline: 97306356 Human tyrosyl-tRNA synthetase shares amino acid sequence homology with a putative cytokine." *J Biol Chem* 1997;272:14420-14425.

[2] Simos G, Segref A, Fasiolo F, Hellmuth K, Shevchenko A, Mann M, Hurt EC; Medline: 97050848 The yeast protein Arc1p binds to tRNA and functions as a cofactor for the methionyl- and glutamyl-tRNA synthetases." *EMBO J* 1996;15:5437-5448.

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906. UbiA prenyltransferase family signature (UbiA)

The following prenyltransferases are evolutionary related [1,2]:

- Bacterial 4-hydroxybenzoate octaprenyltransferase (gene *ubiA*).
 - Yeast mitochondrial para-hydroxybenzoate--polyprenyltransferase (gene *COQ2*).
 - Protoheme IX farnesyltransferase (heme O synthase) from yeast and mammals (gene
- 5 COX10) and from bacteria (genes *cyoE* or *ctaB*).

These proteins probably contain seven transmembrane segments. The best conserved region is located in a loop between the second and third of these segments and was used as a signature pattern.

10 Consensus pattern N-x(3)-[DE]-x(2)-[LIF]-D-x(2)-[VM]-x-R-[ST]-x(2)-R-x(4)-G Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROT NONE.

- 15 [1] Melzer M., Heide L. *Biochim. Biophys. Acta* 1212:93-102(1994).
[2] Mogi T., Saiki K., Anraku Y. *Mol. Microbiol.* 14:391-398(1994).

907. Uncharacterized protein family UPF0044 signature (UPF0044)

- 20 The following uncharacterized proteins have been shown [1] to be highly similar:
- *Bacillus subtilis* hypothetical protein yqeI.
 - *Escherichia coli* hypothetical protein yhbY and HI1333, the corresponding *Haemophilus influenzae* protein.
 - *Methanococcus jannaschii* hypothetical protein MJ0652.

- 25 These are small proteins of 10 to 15 Kd. They can be picked up in the database by the following pattern. This pattern is located in the N-terminal part of these proteins.

Consensus pattern L-[ST]-x(3)-K-x(3)-[KR]-[SGA]-x-[GA]-H-x-L-x-P-[LIV]-x(2)-[LIV]-[GA]-x(2)-G Sequences known to belong to this class detected by the pattern ALL.

30 908. ATP synthase (C/AC39) subunit (vATP-synt_AC39)

This family includes the AC39 subunit from vacuolar ATP synthase Swiss:P32366 [1], and the C subunit from archaeobacterial ATP synthase [2]. The family also includes subunit C

from the Sodium transporting ATP synthase from *Enterococcus hirae* Swiss:P43456 [3].
Number of members: 12

[1] Bauerle C, Ho MN, Lindorfer MA, Stevens TH; Medline: 93286119 The *Saccharomyces cerevisiae* VMA6 gene encodes the 36-kDa subunit of the vacuolar H(+)-ATPase membrane sector." J Biol Chem 1993;268:12749-12757.

[2] Wilms R, Freiberg C, Wegerle E, Meier I, Mayer F, Muller V; Medline: 96324968 Subunit structure and organization of the genes of the A1A0 ATPase from the Archaeon *Methanosarcina mazei* Go1." J Biol Chem 1996;271:18843-18852.

[3] Takase K, Kakinuma S, Yamato I, Konishi K, Igarashi K, Kakinuma Y; Medline: 94209269 Sequencing and characterization of the *ntp* gene cluster for vacuolar- type Na(+)-translocating ATPase of *Enterococcus hirae*." J Biol Chem 1994;269:11037-11044.

909. ATP synthase (E/31 kDa) subunit (vATP-synt_E)
This family includes the vacuolar ATP synthase E subunit [1], as well as the archaeobacterial ATP synthase E subunit [2]. Number of members: 24

[1] Foury F; Medline: 91009356 The 31-kDa polypeptide is an essential subunit of the vacuolar ATPase in *Saccharomyces cerevisiae*." J Biol Chem 1990;265:18554-18560.

[2] Wilms R, Freiberg C, Wegerle E, Meier I, Mayer F, Muller V; Medline: 96324968 Subunit structure and organization of the genes of the A1A0 ATPase from the Archaeon *Methanosarcina mazei* Go1." J Biol Chem 1996;271:18843-18852.

910. (WW)

The WW domain [1-4,E1] (also known as *rsp5* or WWP) has been originally discovered as a short conserved region in a number of unrelated proteins, among them dystrophin, the gene responsible for Duchenne muscular dystrophy. The domain, which spans about 35 residues, is repeated up to 4 times in some proteins. It has been shown [5] to bind proteins with particular proline- motifs, [AP]-P-P-[AP]-Y, and thus resembles somewhat SH3 domains. It appears to contain beta-strands grouped around four conserved aromatic positions; generally Trp. The name WW or WWP derives from the presence of these Trp as well as that of a conserved Pro.

It is frequently associated with other domains typical for proteins in signal transduction processes.

Proteins containing the WW domain are listed below.

- 5 - Dystrophin, a multidomain cytoskeletal protein. Its longest alternatively spliced form consists of an N-terminal actin-binding domain, followed by 24 spectrin-like repeats, a cysteine-rich calcium-binding domain and a C-terminal globular domain. Dystrophin forms tetramers and is thought to have multiple functions including involvement in membrane stability, transduction of contractile forces to the extracellular environment and organization of membrane specialization. Mutations in the dystrophin gene lead to muscular dystrophy of Duchenne or Becker type. Dystrophin contains one WW domain C-terminal of the spectrin-repeats.
- 10 - Utrophin, a dystrophin-like protein of unknown function.
- Vertebrate YAP protein is a substrate of an unknown serine kinase. It binds to the SH3 domain of the Yes oncoprotein via a proline-rich region. This protein appears in alternatively spliced isoforms, containing either one or two WW domains [6].
- 15 - Mouse NEDD-4 plays a role in the embryonic development and differentiation of the central nervous system. It contains 3 WW modules followed by a HECT domain. The human ortholog contains 4 WW domains, but the third WW domain is probably spliced resulting in an alternate NEDD-4 protein with only 3 WW modules [3].
- 20 - Yeast RSP5 is similar to NEDD-4 in its molecular organization. It contains an N-terminal C2 domain (see <PDOC00380>), followed by a histidine-rich region, 3 WW domains and a HECT domain.
- Rat FE65, a transcription-factor activator expressed preferentially in liver. The activator domain is located within the N-terminal 232 residues of FE65, which also contain the WW domain.
- 25 - Yeast ESS1/PTF1, a putative peptidyl prolyl cis-trans isomerase from family ppiC (see <PDOC00840>). A related protein, dodo (gene dod) exists in Drosophila and in mammals (gene PIN1).
- 30 - Tobacco DB10 protein. The WW domain is located N-terminal to the region with similarity to ATP-dependent RNA helicases.
- IQGAP, a human GTPase activating protein acting on ras. It contains an N-terminal domain similar to fly muscle mp20 protein and a C-terminal ras GTPase activator domain.

- Yeast pre-mRNA processing protein PRP40, *Caenorhabditis elegans* ZK1098.1 and fission yeast SpAC13C5.02 are related proteins with similarity to MYO2- type myosin, each containing two WW-domains at the N-terminus.

- *Caenorhabditis elegans* hypothetical protein C38D4.5, which contains one WW module, a PH domain (see <PDOC50003>) and a C-terminal phosphatidylinositol 3-kinase domain.

- Yeast hypothetical protein YFL010c.

For the sensitive detection of WW domains, a profile was developed which spans the whole homology region as well as a pattern.

- 10 Consensus pattern W-x(9,11)-[VFY]-[FYW]-x(6,7)-[GSTNE]-[GSTOCR]-[FYW]-x(2)-P
Sequences known to belong to this class detected by the pattern ALL. Other sequence(s)
detected in SWISS-PROT8. Sequences known to belong to this class detected by the
profile ALL.

- 15 [1] Bork P., Sudol M. Trends Biochem. Sci. 19:531-533(1994).
[2] Andre B., Springael J.Y. Biochem. Biophys. Res. Commun. 205:1201-1205(1994).
[3] Hofmann K.O., Bucher P. FEBS Lett. 358:153-157(1995).
[4] Sudol M., Chen H.I., Bougeret C., Einbond A., Bork P. FEBS Lett. 369:67-71(1995).
[5] Chen H.I., Sudol M. Proc. Natl. Acad. Sci. U.S.A. 92:7819-7823(1995).
20 [6] Sudol M., Bork P., Einbond A., Kastury K., Druck T., Negrini M., Huebner K., Lehman
D. J. Biol. Chem. 270:14733-14741(1995).

911. Xeroderma pigmentosum (XP) [1] (XPG_1)

- 25 Xeroderma pigmentosum (XP) [1] is a human autosomal recessive disease, characterized by a
high incidence of sunlight-induced skin cancer. People's skin cells with this condition are
hypersensitive to ultraviolet light, due to defects in the incision step of DNA excision repair.
There are a minimum of seven genetic complementation groups involved in this pathway:
XP-A to XP-G. The defect in XP-G can be corrected by a 133 Kd nuclear protein called XPG
30 (or XPGC) [2].

XPG belongs to a family of proteins [2,3,4,5,6] that are composed of two main subsets:

- Subset 1, to which belongs XPG, RAD2 from budding yeast and rad13 from fission yeast. RAD2 and XPG are single-stranded DNA endonucleases [7,8]. XPG makes the 3' incision in human DNA nucleotide excision repair [9].
- Subset 2, to which belongs mouse and human FEN-1, rad2 from fission yeast, and RAD27 from budding yeast. FEN-1 is a structure-specific endonuclease.

In addition to the proteins listed in the above groups, this family also includes:

- Fission yeast *exo1*, a 5'->3' double-stranded DNA exonuclease that could act in a pathway that corrects mismatched base pairs.
- Yeast EXO1 (DHS1), a protein with probably the same function as *exo1*.
- Yeast DIN7.

- Sequence alignment of this family of proteins reveals that similarities are largely confined to two regions. The first is located at the N-terminal extremity (N-region) and corresponds to the first 95 to 105 amino acids. The second region is internal (I-region) and found towards the C-terminus; it spans about 140 residues and contains a highly conserved core of 27 amino acids that includes a conserved pentapeptide (E-A-[DE]-A-[QS]). It is possible that the conserved acidic residues are involved in the catalytic mechanism of DNA excision repair in XPG. The amino acids linking the N- and I-regions are not conserved; indeed, they are largely absent from proteins belonging to the second subset.

Two signature patterns were developed for these proteins. The first corresponds to the central part of the N-region, the second to part of the I-region and includes the putative catalytic core pentapeptide.

- Consensus pattern [VI]-[KRE]-P-x-[FYIL]-V-F-D-G-x(2)-[PIL]-x-[LVC]-K Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROT NONE.
- Consensus pattern [GS]-[LIVM]-[PER]-[FYS]-[LIVM]-x-A-P-x-E-A-[DE]-[PAS]-[OS]-[CLM] Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROT NONE.

[2] Scherly D., Nouspikel T., Corlet J., UCLA C., Bairoch A., Clarkson S.G. Nature 363:182-185(1993).

[3] Carr A.M., Sheldrick K.S., Murray J.M., Al-Harithy R., Watts F.Z., Lehmann A.R. Nucleic Acids Res. 21:1345-1349(1993).

5 [4] Murray J.M., Tavassoli M., Al-Harithy R., Sheldrick K.S., Lehmann A.R., Carr A.M., Watts F.Z. Mol. Cell. Biol. 14:4878-4888(1994).

[5] Harrington J.J., Lieber M.R. Genes Dev. 8:1344-1355(1994).

[6] Szankasi P., Smith G.R. Science 267:1166-1169(1995).

[7] Habraken Y., Sung P., Prakash L., Prakash S. Nature 366:365-368(1993).

10 [8] O'Donovan A., Scherly D., Clarkson S.G., Wood R.D. J. Biol. Chem. 269:15965-15968(1994).

[9] O'Donovan A., Davies A.A., Moggs J.G., West S.C., Wood R.D. Nature 371:432-435(1994).

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912. 5-formyltetrahydrofolate cyclo-ligase (5-FTHF_cyc-lig)

5-formyltetrahydrofolate cyclo-ligase or methenyl-THF synthetase EC:6.3.3.2 catalyses the interchange of 5-formyltetrahydrofolate (5-FTHF) to 5-10-methenyltetrahydrofolate, this requires ATP and Mg²⁺ [1]. 5-FTHF is used in chemotherapy where it is clinically known as Leucovorin [2].

20

Number of members: 23

[1] Dayan A, Bertrand R, Beauchemin M, Chahla D, Mamo A, Filion M, Skup D, Massie B,

25

Jolivet J; Medline: 96096540 Cloning and characterization of the human 5,10-methenyltetrahydrofolate synthetase-encoding cDNA." Gene 1995;165:307-311.

[2] Maras B, Stover P, Valiante S, Barra D, Schirch V; Medline: 94308074 Primary structure and tetrahydropteroylglutamate binding site of rabbit liver cytosolic 5,10-methenyltetrahydrofolate synthetase." J Biol Chem 1994;269:18429-18433.

30

913. Cytosolic long-chain acyl-CoA thioester hydrolase (Acyl-CoA_hydro)

This family consist of various cytosolic long-chain acyl-CoA thioester hydrolases including human and rat [1,2]. The aligned region is repeated with in the sequence of human and rat

cytosolic long-chain acyl-CoA thioester hydrolases of this family. Long-chain acyl-CoA hydrolases hydrolyse palmitoyl-CoA to CoA and palmitate, they also catalyse the hydrolysis of other long chain fatty acyl-CoA thioesters. Long-chain acyl-CoA hydrolases are present in all living organisms and they may provide a mechanism for the control of lipid metabolism

5 [1].

Number of members: 24

10 [1]Yamada J, Furihata T, Iida N, Watanabe T, Hosokawa M, Satoh T, Someya A, Nagaoka I, Suga T; Medline: 97236308 Molecular cloning and expression of cDNAs encoding rat brain and liver cytosolic long-chain acyl-CoA hydrolases." Biochem Biophys Res Commun 1997;232:198-203.

[2] Broustas CG, Larkins LK, Uhler MD, Hajra AK; Medline: 96209964 Molecular cloning and expression of cDNA encoding rat brain cytosolic acyl-coenzyme A thioester hydrolase." J Biol Chem 1996;271:10470-10476.

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914. Agglutinin

Lectin (probable mannose binding)

Members of this family are plant lectins. Many if not all are mannose specific.

20

Number of members: 87

[1] Wright CS, Hester G; Medline: 97094989 The 2.0 Å structure of a cross-linked complex between snowdrop lectin and a branched mannopentaose: evidence for two unique binding modes." Structure 1996;4:1339-1352.

25

915. (ANF_RECEPTORS)

Natriuretic peptides are hormones involved in the regulation of fluid and electrolyte homeostasis. These hormones stimulate the intracellular production of cyclic GMP as a second messenger.

30

Currently, three types of natriuretic peptide receptors are known [1,2]. Two express guanylate cyclase activity: GC-A (or ANP-A) which seems specific to atrial natriuretic peptide (ANP), and GC-B (or ANP-B) which seems to be stimulated more effectively by brain natriuretic

peptide (BNP) than by ANP. The third receptor (ANP-C) is probably responsible for the clearance of ANP from the circulation and does not play a role in signal transduction.

GC-A and GC-B are plasma membrane-bound proteins that share the following topology: an N-terminal extracellular domain which acts as the ligand binding region, then a transmembrane domain followed by a large cytoplasmic C- terminal region that can be subdivided into two domains: a protein kinase-like domain (see <PDOC00100>) that appears important for proper signalling and a guanylate cyclase catalytic domain (see <PDOC00425>). The topology of ANP-C is different: like GC-A and -B it possesses an extracellular ligand-binding region and a transmembrane domain, but its cytoplasmic domain is very short.

A pattern was developed from the ligand-binding region of natriuretic peptide receptors based on a highly conserved region located in the N-terminal part of the domain.

Consensus patternG-P-x-C-x-Y-x-A-A-x-V-x-R-x(3)-H-W Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROT NONE.

[1] Garbers D.L. New Biol. 2:499-504(1990).

[2] Schulz S., Chinkers M., Garbers D.L. FASEB J. 2:2026-2035(1989).

916. (Apocytochrome)

Cytochrome c family heme-binding site signature

In proteins belonging to cytochrome c family [1], the heme group is covalently attached by thioether bonds to two conserved cysteine residues. The consensus sequence for this site is Cys-X-X-Cys-His and the histidine residue is one of the two axial ligands of the heme iron. This arrangement is shared by all proteins known to belong to cytochrome c family, which presently includes cytochromes c, c', c1 to c6, c550 to c556, cc3/Hmc, cytochrome f and reaction center cytochrome c.

Consensus patternC-{CPWHF}-{CPWR}-C-H-{CFYW} Sequences known to belong to this class detected by the patternALL, except for four cytochrome c's which lack the first thioether bond. Other sequence(s) detected in SWISS-PROT454.

Note: some cytochrome c's have more than a single bound heme group c4 has 2, c7 has 3, c3 has 4, the reaction center has 4, and cc3/Hmc has 16 !

- 5 [1] Mathews F.S. Prog. Biophys. Mol. Biol. 45:1-56(1985).

917. ATP-synt_A-c. ATP synthase Alpha chain, C terminal

[1] Medline: 94344236. Structure at 2.8 A resolution of F1-ATPase from bovine heart mitochondria. Abrahams JP, Leslie AG, Lutter R, Walker JE; Nature 1994;370:621-628.

- 10 Number of members: 125

918. (Basic)

Myc-type, 'helix-loop-helix' dimerization domain signature

HELIX_LOOP_HELIX

15

A number of eukaryotic proteins, which probably are sequence specific DNA-binding proteins that act as transcription factors, share a conserved domain of 40 to 50 amino acid residues. It has been proposed [1] that this domain is formed of two amphipathic helices joined by a variable length linker region that could form a loop. This 'helix-loop-helix' (HLH) domain mediates protein dimerization and has been found in the proteins listed below [2,3,E1,E2]. Most of these proteins have an extra basic region of about 15 amino acid residues that is adjacent to the HLH domain and specifically binds to DNA. They are referred as basic helix-loop-helix proteins (bHLH), and are classified in two groups: class A (ubiquitous) and class B (tissue-specific). Members of the bHLH family bind variations on the core sequence 'CANNTG', also referred to as the E-box motif. The homo- or heterodimerization mediated by the HLH domain is independent of, but necessary for DNA binding, as two basic regions are required for DNA binding activity. The HLH proteins lacking the basic domain (Emc, Id) function as negative regulators since they form heterodimers, but fail to bind DNA. The hairy-related proteins (hairy, E(spl), deadpan) also repress transcription although they can bind DNA. The proteins of this subfamily act together with co-repressor proteins, like groucho, through their C-terminal motif WRPW.

30

- The myc family of cellular oncogenes [4], which is currently known to contain four members: c-myc [E3], N-myc, L-myc, and B-myc. The myc genes are thought to play a role in cellular differentiation and proliferation.

- Proteins involved in myogenesis (the induction of muscle cells). In mammals MyoD1 (Myf-3), myogenin (Myf-4), Myf-5, and Myf-6 (Mrf4 or herculin), in birds CMD1 (QMF-1), in *Xenopus* MyoD and MF25, in *Caenorhabditis elegans* CeMyoD, and in *Drosophila nautilus* (nau).
- 5 - Vertebrate proteins that bind specific DNA sequences ('E boxes') in various immunoglobulin chains enhancers: E2A or ITF-1 (E12/pan-2 and E47/pan-1), ITF-2 (tcf4), TFE3, and TFE8.
- Vertebrate neurogenic differentiation factor 1 that acts as differentiation factor during neurogenesis.
- 10 - Vertebrate MAX protein, a transcription regulator that forms a sequence- specific DNA-binding protein complex with myc or mad.
- Vertebrate Max Interacting Protein 1 (MXI1 protein) which acts as a transcriptional repressor and may antagonize myc transcriptional activity by competing for max.
- Proteins of the bHLH/PAS superfamily which are transcriptional activators. In mammals,
- 15 AH receptor nuclear translocator (ARNT), single-minded homologs (SIM1 and SIM2), hypoxia-inducible factor 1 alpha (HIF1A), AH receptor (AHR), neuronal pas domain proteins (NPAS1 and NPAS2), endothelial pas domain protein 1 (EPAS1), mouse ARNT2, and human BMAL1. In *drosophila*, single-minded (SIM), AH receptor nuclear translocator (ARNT), trachealess protein (TRH), and similar protein (SIMA).
- 20 - Mammalian transcription factors HES, which repress transcription by acting on two types of DNA sequences, the E box and the N box.
- Mammalian MAD protein (max dimerizer) which acts as transcriptional repressor and may antagonize myc transcriptional activity by competing for max.
- Mammalian Upstream Stimulatory Factor 1 and 2 (USF1 and USF2), which bind to a
- 25 symmetrical DNA sequence that is found in a variety of viral and cellular promoters.
- Human lyl-1 protein; which is involved, by chromosomal translocation, in T- cell leukemia.
- Human transcription factor AP-4.
- Mouse helix-loop-helix proteins MATH-1 and MATH-2 which activate E box- dependent transcription in collaboration with E47.
- 30 - Mammalian stem cell protein (SCL) (also known as tal1), a protein which may play an important role in hemopoietic differentiation. SCL is involved, by chromosomal translocation, in stem-cell leukemia.

- Mammalian proteins Id1 to Id4 [5]. Id (inhibitor of DNA binding) proteins lack a basic DNA-binding domain but are able to form heterodimers with other HLH proteins, thereby inhibiting binding to DNA.
- Drosophila extra-macrochaetae (emc) protein, which participates in sensory organ
- 5 patterning by antagonizing the neurogenic activity of the achaete- scute complex. Emc is the homolog of mammalian Id proteins.
- Human Sterol Regulatory Element Binding Protein 1 (SREBP-1), a transcriptional activator that binds to the sterol regulatory element 1 (SRE-1) found in the flanking region of the LDLR gene and in other genes.
- 10 - Drosophila achaete-scute (AS-C) complex proteins T3 (l'sc), T4 (scute), T5 (achaete) and T8 (asense). The AS-C proteins are involved in the determination of the neuronal precursors in the peripheral nervous system and the central nervous system.
- Mammalian homologs of achaete-scute proteins, the MASH-1 and MASH-2 proteins.
- Drosophila atonal protein (ato) which is involved in neurogenesis.
- 15 - Drosophila daughterless (da) protein, which is essential for neurogenesis and sex-determination.
- Drosophila deadpan (dpn), a hairy-like protein involved in the functional differentiation of neurons.
- Drosophila delilah (dei) protein, which is plays an important role in the differentiation of
- 20 epidermal cells into muscle.
- Drosophila hairy (h) protein, a transcriptional repressor which regulates the embryonic segmentation and adult bristle patterning.
- Drosophila enhancer of split proteins E(spl), that are hairy-like proteins active during neurogenesis. also act as transcriptional repressors.
- 25 - Drosophila twist (twi) protein, which is involved in the establishment of germ layers in embryos.
- Maize anthocyanin regulatory proteins R-S and LC.
- Yeast centromere-binding protein 1 (CPF1 or CBF1). This protein is involved in chromosomal segregation. It binds to a highly conserved DNA sequence, found in centromers
- 30 and in several promoters.
- Yeast INO2 and INO4 proteins.
- Yeast phosphate system positive regulatory protein PHO4 which interacts with the upstream activating sequence of several acid phosphatase genes.
- Yeast serine-rich protein TYE7 that is required for ty-mediated ADH2 expression.

degree of variability and only a small number of residues are conserved in addition to the catalytic serine.

Since a pattern detecting all serine beta-lactamases would also pick up many unrelated sequences, it was decided to provide specific patterns, centered on the active site serine, for each of the three classes.

Consensus pattern [FY]-x-[LIVMFY]-x-S-[TV]-x-K-x(4)-[AGLM]-x(2)-[LC] [S is the active site residue] Sequences known to belong to this class detected by the patternALL class-A beta-lactamases. Other sequence(s) detected in SWISS-PROT7.

Consensus pattern F-E-[LIVM]-G-S-[LIVMG]-[SA]-K [The first S is the active site residue] Sequences known to belong to this class detected by the patternALL class-C beta-lactamases. Other sequence(s) detected in SWISS-PROTNONE.

Consensus pattern [PA]-x-S-[ST]-F-K-[LIV]-[PAL]-x-[STA]-[LI] [S is the active site residue] Sequences known to belong to this class detected by the patternALL class-D beta-lactamases. Other sequence(s) detected in SWISS-PROTNONE.

- [1] Ambler R.P. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 289:321-331(1980).
- [2] Pastor N., Pinero D., Valdes A.M., Soberon X. Mol. Microbiol. 4:1957-1965(1990).
- [3] Bush K. Antimicrob. Agents Chemother. 33:259-263(1989).
- [4] Joris B., Ghuysen J.-M., Dive G., Renard A., Dideberg O., Charlier P., Frere J.M., Kelly J.A., Boyington J.C., Moews P.C., Knox J.R. Biochem. J. 250:313-324(1988).

920. Biotin protein ligase (BPL)

Biotin is covalently attached at the active site of certain enzymes that transfer carbon dioxide from bicarbonate to organic acids to form cellular metabolites. Biotin protein ligase (BPL) is the enzyme responsible for attaching biotin to a specific lysine at the active site of biotin enzymes. Each organism probably has only one BPL. Biotin attachment is a two step reaction that results in the formation of an amide linkage between the carboxyl group of biotin and the epsilon-amino group of the modified lysine [2].

Number of members: 26

[1] Wilson KP, Shewchuk LM, Brennan RG, Otsuka AJ, Matthews BW; Medline: 93028443
Escherichia coli biotin holoenzyme synthetase/bio repressor crystal structure delineates the
biotin- and DNA-binding domains." Proc Natl Acad Sci USA 1992;89:9257-9261.

- 5 [2] Chapman-Smith A, Cronan JE Jr; Medline: 10470036 The enzymatic biotinylation of
proteins: a post-translational modification of exceptional specificity." Trends Biochem Sci
1999;24:359-363.

921. (BRCA2_repeat)

10

The alignment covers only the most conserved region of the repeat. Respiratory-chain NADH
dehydrogenase 30 Kd subunit signature

15

[1] Bork P, Blomberg N, Nilges M; Medline: 96241568 Internal repeats in the BRCA2
protein sequence." Nat Genet 1996;13:22-23.

Number of members: 63

20

922. (C6)

This domain of unknown function is found in the C. elegans protein Swiss:Q19522. It is
presumed to be an extracellular domain. The C6 domain contains six conserved cysteine
residues in most copies of the domain. However some copies of the domain are missing
cysteine residues 1 and 3 suggesting that these form a disulphide bridge.

25

Number of members: 23

923. Cadherin cytoplasmic region (Cadherin_C_term)

30

Cadherins are vital in cell-cell adhesion during tissue differentiation. Cadherins are linked to
the cytoskeleton by catenins. Catenins bind to the cytoplasmic tail of the cadherin. Cadherins
cluster to form foci of homophilic binding units. A key determinant to the strength of the
binding that it is mediated by cadherins is the juxtamembrane region of the cadherin. This
region induces clustering and also binds to the protein p120ctn [1].

Number of members: 59

[1] Yap AS, Niessen CM, Gumbiner BM; Medline: 98234411 The juxtamembrane region of the cadherin cytoplasmic tail supports lateral clustering, adhesive strengthening, and interaction with p120ctn." J Cell Biol 1998;141:779-789.

5 [2] Barth AI, Nathke IS, Nelson WJ; Medline: 97471931 Cadherins, catenins and APC protein: interplay between cytoskeletal complexes and signaling pathways." Curr Opin Cell Biol 1997;9:683-690.

[3] Braga VM, Machesky LM, Hall A, Hotchin NA; Medline: 97327766 The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts." J

10 Cell Biol 1997;137:1421-1431.

924. Clathrin propeller repeat (Clathrin_propel)

Clathrin is the scaffold protein of the basket-like coat that surrounds coated vesicles. The soluble assembly unit, a triskelion, contains three heavy chains and three light chains in an extended three-legged structure. Each leg contains one heavy and one light chain. The N-terminus of the heavy chain is known as the globular domain, and is composed of seven repeats which form a beta propeller [1].

Number of members: 61

[1] ter Haar E, Musacchio A, Harrison SC, Kirchhausen T; Medline: 99043510 Atomic structure of clathrin: a beta propeller terminal domain joins an alpha zigzag linker." Cell. 1998;95:563-573.

25 925. Respiratory-chain NADH dehydrogenase 30 Kd subunit signature (complex1_30Kd)

Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there is one with a molecular weight of 30 Kd (in mammals) which has been found to be:

- Nuclear encoded, as a precursor form with a transit peptide in mammals, and in *Neurospora crassa*.

- Mitochondrial encoded in *Paramecium* (protein P1), and in the slime mold *Dictyostelium discoideum* (ORF 209).

- Chloroplast encoded in various higher plants (ORF 159). It is also present in bacteria:

- In the cyanobacteria *Synechocystis* strain PCC 6803 (gene *ndhJ*).

5 - Subunit C of *Escherichia coli* NADH-ubiquinone oxidoreductase (gene *nuoC*).

- Subunit NQO5 of *Paracoccus denitrificans* NADH-ubiquinone oxidoreductase.

This protein, in its mature form, consists of from 157 to 266 amino acid residues. The best conserved region is located in the C-terminal section and can be used as a signature pattern.

10

Consensus pattern E-R-E-x(2)-[DE]-[LIVMFY](2)-x(6)-[HK]-x(3)-[KRP]-x-[LIVM]-[LIVMYS] Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE.

15 [1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).

[2] Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).

926. Respiratory-chain NADH dehydrogenase 49 Kd subunit signature (complex1_49Kd)

20 Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there is one with a molecular weight of 49 Kd

25 (in mammals), which is the third largest subunit of complex I and is a component of the iron-sulfur (IP) fragment of the enzyme. It seems to bind a 4Fe-4S iron-sulfur cluster. The 49 Kd subunit has been found to be:

- Nuclear encoded, as a precursor form with a transit peptide in mammals, and in *Neurospora crassa*.

30 - Mitochondrial encoded in protozoan such as *Paramecium* (ORF 400), *Leishmania* and *Trypanosoma* (MURF 3).

- Chloroplast encoded in various higher plants (ORF 392).

The 49 Kd subunit is highly similar to [3,4]:

- Subunit D of *Escherichia coli* NADH-ubiquinone oxidoreductase (gene *nuoD*).

- Subunit NQO4 of *Paracoccus denitrificans* NADH-ubiquinone oxidoreductase.
- Subunit 5 of *Escherichia coli* formate hydrogenlyase (gene hycE).
- Subunit G of *Escherichia coli* hydrogenase-4 (gene hyfG).

A highly conserved region was selected as signature pattern, located in the N-terminal section of this subunit.

Consensus pattern [LIVMH]-H-[RT]-[GA]-x-E-K-[LIVMTN]-x-E-x-[KRQ] Sequences known to belong to this class detected by the patternALL.

[1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).

[2] Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).

[3] Fearnley I.M., Walker J.E. Biochim. Biophys. Acta 1140:105-134(1992).

[4] Weidner U., Geier S., Ptöck A., Friedrich T., Leif H., Weiss H. J. Mol. Biol. 233:109-122(1993).

927. (COX2)

Cytochrome c oxidase (EC 1.9.3.1) [1,2] is an oligomeric enzymatic complex which is a component of the respiratory chain and is involved in the transfer of electrons from cytochrome c to oxygen. In eukaryotes this enzyme complex is located in the mitochondrial inner membrane; in aerobic prokaryotes it is found in the plasma membrane. The enzyme complex consists of 3-4 subunits (prokaryotes) to up to 13 polypeptides (mammals).

Subunit 2 (CO II) transfers the electrons from cytochrome c to the catalytic subunit 1. It contains two adjacent transmembrane regions in its N-terminus and the major part of the protein is exposed to the periplasmic or to the mitochondrial intermembrane space, respectively. CO II provides the substrate-binding site and contains a copper center called Cu(A), probably the primary acceptor in cytochrome c oxidase. An exception is the corresponding subunit of the cbb3-type oxidase which lacks the copper A redox-center. Several bacterial CO II have a C-terminal extension that contains a covalently bound heme c.

It has been shown [3,4] that nitrous oxide reductase (EC 1.7.99.6) (gene nosZ) of *Pseudomonas* has sequence similarity in its C-terminus to CO II. This enzyme is part of the bacterial respiratory system which is activated under anaerobic conditions in the presence of

nitrate or nitrous oxide. NosZ is a periplasmic homodimer that contains a dinuclear copper center, probably located in a 3- dimensional fold similar to the cupredoxin-like fold that has been suggested for the copper-binding site of CO II [3].

- 5 The dinuclear purple copper center is formed by 2 histidines and 2 cysteines [5]. This region was used as a signature pattern. The conserved valine and the conserved methionine are said to be involved in stabilizing the copper-binding fold by interacting with each other.

Consensus pattern V-x-H-x(33,40)-C-x(3)-C-x(3)-H-x(2)-M [The two C's and two H's are
10 copper ligands] Sequences known to belong to this class detected by the patternALL, except for *Paramecium primaurelia* as well as in some plants where the pattern ends with Thr; an RNA editing event at this position could change this Thr to Met.

Note: cytochrome cbb(3) subunit 2 does not belong to this family.

- 15 [1] Capaldi R.A., Malatesta F., Darley-Usmar V.M. *Biochim. Biophys. Acta* 726:135-148(1983).
- [2] Garcia-Horsman J.A., Barquera B., Rumbley J., Ma J., Gennis R.B. *J. Bacteriol.* 176:5587-5600(1994).
- 20 [3] van der Oost J., Lappalainen P., Musacchio A., Warne A., Lemieux L., Rumbley J., Gennis R.B., Aasa R., Pascher T., Malmstrom B.G., Saraste M. *EMBO J.* 11:3209-3217(1992).
- [4] Zumft W.G., Dreusch A., Loechele S., Cuypers H., Friedrich B., Schneider B. *Eur. J. Biochem.* 208:31-40(1992).

25 928. Cytochrome C assembly protein (CytC_asm)

This family consists of various proteins involved in cytochrome c assembly from mitochondria and bacteria: CycK from *Rhizobium* [3], CcmC from *E. coli* and *Paracoccus*
30 denitrificans [2,1] and orf240 from wheat mitochondria [4]. The members of this family are probably integral membrane proteins with six predicted transmembrane helices. It has been proposed that members of this family comprise a membrane component of an ABC (ATP binding cassette) transporter complex. It is also proposed that this transporter is necessary for transport of some component needed for cytochrome c assembly. One member CycK

contains a putative heme-binding motif [3], orf240 also contains a putative heme-binding motif and is a proposed ABC transporter with c-type heme as its proposed substrate [4]. However it seems unlikely that all members of this family transport heme nor c-type apocytochromes because CcmC in the putative CcmABC transporter transports neither [1].

5 Number of members: 67

[1] Page D, Pearce DA, Norris HA, Ferguson SJ; Medline: 97195802 The *Paracoccus denitrificans* ccmA, B and C genes: cloning and sequencing, and analysis of the potential of their products to form a haem or apo-c-type cytochrome transporter. MICROBIOLOGY

10 1997;143:563-576.

[2] Thoeny-meyer L, Fischer F, Kunzler P, Ritz D, Hennecke H; Medline: 95362656

Escherichia coli genes required for cytochrome c maturation." J. BACTERIOL

1995;177:4321-4326.

[3] Delgado MJ, Yeoman KH, Wu G, Vargas C, Davies A, Poole RK, Johnston AWB,

15 Downie JA; Medline: 95394794 Characterization of the cychIJKL genes involved in cytochrome c biogenesis and symbiotic nitrogen fixation in *Rhizobium leguminosarum*." J. BACTERIOL 1995;177:4927-4934.

[4] Bonnard G, Grienemberger JM; Medline: 95124303 A gene proposed to encode a transmembrane domain of an ABC transporter is expressed in wheat mitochondria." MOL.

20 GEN. GENET 1995;246:91-99.

929. Cytochrome b559 subunits heme-binding site signature (cytochr_b559)

Cytochrome b559 [1] is an essential component of photosystem II complex from oxygenic
25 photosynthetic organisms. It is an integral thylakoid membrane protein composed of two subunits, alpha (gene psbE) and beta (gene psbF), each of which contains a histidine residue located in a transmembrane region. The two histidines coordinate the heme iron of cytochrome b559.

30 The region around the heme-binding residue of both subunits is very similar and can be used as a signature pattern.

Consensus pattern[LIV]-x-[ST]-[LIVF]-R-[FYW]-x(2)-[IV]-H-[STGA]-[LIV]-[STGA]-[IV]-P [H is the heme iron ligand] Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROT/NONE.

- 5 [1] Pakrasi H.B., de Ciechi P., Whitmarsh J. EMBO J. 10:1619-1627(1991).

930. Cytochrome b/b6 signatures (Cytochrome_b)

- 10 In the mitochondrion of eukaryotes and in aerobic prokaryotes, cytochrome b is a component of respiratory chain complex III (EC 1.10.2.2) - also known as the bc₁ complex or ubiquinol-cytochrome c reductase. In plant chloroplasts and cyanobacteria, there is an analogous protein, cytochrome b₆, a component of the plastoquinone-plastocyanin reductase (EC 1.10.99.1), also known as the b₆f complex.

- 15 Cytochrome b/b₆ [1,2] is an integral membrane protein of approximately 400 amino acid residues that probably has 8 transmembrane segments. In plants and cyanobacteria, cytochrome b₆ consists of two subunits encoded by the petB and petD genes. The sequence of petB is colinear with the N-terminal part of mitochondrial cytochrome b, while petD corresponds to the C-terminal part. Cytochrome b/b₆ non-covalently binds two heme groups, known as b₅₆₂ and b₅₆₆. Four conserved histidine residues are postulated to be the ligands of the iron atoms of these two heme groups.

- 25 Apart from regions around some of the histidine heme ligands, there are a few conserved regions in the sequence of b/b₆. The best conserved of these regions includes an invariant P-E-W triplet which lies in the loop that separates the fifth and sixth transmembrane segments. It seems to be important for electron transfer at the ubiquinone redox site - called Q_z or Q_o (where o stands for outside) - located on the outer side of the membrane.

- 30 A schematic representation of the structure of cytochrome b/b₆ is shown below.

```

+---Fe-b562---+ | +---Fe-b566---|+ |||
xxxxxxxxxxHxHxxxxxxxxxxHxHxxxxxxxxxxPEWxxxxxxxxxxxxxxxxxxxx <-----

```

---Cytochrome-b-----> <----Cytochrome-b6-petB-----><--Cytochrome-
b6-petD----->

Two signature patterns were developed for cytochrome b/b6. The first includes the first
5 conserved histidine of b/b6, which is a heme b562 ligand; the second includes the conserved
PEW triplet.

Consensus pattern [DENQ]-x(3)-G-[FYWMQ]-x-[LIVMF]-R-x(2)-H [H is a heme b562
ligand] Sequences known to belong to this class detected by the patternALL, except for 5
10 sequences.

Consensus pattern P-[DE]-W-[FY]-[LFY](2) Sequences known to belong to this class
detected by the patternALL, except for *Odocoileus hemionus* (mule deer) and *Paramecium*
15 *tetraurelia* cytochrome b.

[1] Howell N. J. Mol. Evol. 29:157-169(1989).

[2] Esposti M.D., de Vries S., Crimi M., Ghelli A., Patarnello T., Meyer A. Biochim.
Biophys. Acta 1143:243-271(1993).

20 931. Phorbol esters / diacylglycerol binding domain (DAG_PE-bind)

Diacylglycerol (DAG) is an important second messenger. Phorbol esters (PE) are analogues
of DAG and potent tumor promoters that cause a variety of physiological changes when
administered to both cells and tissues. DAG activates a family of serine/threonine protein
25 kinases, collectively known as protein kinase C (PKC) [1]. Phorbol esters can directly
stimulate PKC. The N- terminal region of PKC, known as C1, has been shown [2] to bind PE
and DAG in a phospholipid and zinc-dependent fashion. The C1 region contains one or two
copies (depending on the isozyme of PKC) of a cysteine-rich domain about 50 amino acid
residues long and essential for DAG/PE-binding. Such a domain has also been found in the
30 following proteins:

- Diacylglycerol kinase (EC 2.7.1.107) (DGK) [3], the enzyme that converts DAG into
phosphatidate. It contains two copies of the DAG/PE-binding domain in its N-terminal
section. At least five different forms of DGK are known in mammals.

- N-chimaerin. A brain specific protein which shows sequence similarities with the BCR protein at its C-terminal part and contains a single copy of the DAG/PE-binding domain at its N-terminal part. It has been shown [4,5] to be able to bind phorbol esters.

- The raf/mil family of serine/threonine protein kinases. These protein kinases contain a single N-terminal copy of the DAG/PE-binding domain.

- The unc-13 protein from *Caenorhabditis elegans*. Its function is not known but it contains a copy of the DAG/PE-binding domain in its central section and has been shown to bind specifically to a phorbol ester in the presence of calcium [6].

- The vav oncogene. Vav was generated by a genetic rearrangement during gene transfer assays. Its expression seems to be restricted to cells of hematopoietic origin. Vav seems [5,7] to contain a DAG/PE-binding domain in the central part of the protein.

- The *Drosophila* GTPase activating protein rotund.

The DAG/PE-binding domain binds two zinc ions; the ligands of these metal ions are probably the six cysteines and two histidines that are conserved in this domain. A signature pattern was developed that spans completely the DAG/PE domain.

Consensus pattern H-x-[LIVMFYW]-x(8,11)-C-x(2)-C-x(3)-[LIVMFC]-x(5,10)- C-x(2)-C-x(4)-[HD]-x(2)-C-x(5,9)-C [All the C and H are involved in binding Zinc] Sequences known to belong to this class detected by the pattern ALL, except a few DGK's.

[1] Azzi A., Boscoboinik D., Hensley C. Eur. J. Biochem. 208:547-557(1992).

[2] Ono Y., Fujii T., Igarashi K., Kuno T., Tanaka C, Kikkawa U., Nishizuka Y. Proc. Natl. Acad. Sci. U.S.A. 86:4868-4871(1989).

[3] Sakane F., Yamada K., Kanoh H., Yokoyama C., Tanabe T. Nature 344:345-348(1990).

[4] Ahmed S., Kozma R., Monfries C., Hall C., Lim H.H., Smith P., Lim L. Biochem. J. 272:767-773(1990).

[5] Ahmed S., Kozma R., Lee J., Monfries C., Harden N., Lim L. Biochem. J. 280:233-241(1991).

[6] Ahmed S., Maruyama I.N., Kozma R., Lee J., Brenner S., Lim L. Biochem. J. 287:995-999(1992).

[7] Boguski M.S., Bairoch A., Attwood T.K., Michaels G.S. Nature 358:113-113(1992).

932. 3-dehydroquinate synthase (DHQ_synthase)

[1] Barten R, Meyer TF; Medline: 98273626 Cloning and characterisation of the *Neisseria gonorrhoeae* *aroB* gene." *Mol Gen Genet* 1998;258:34-44.

- [2] Hawkins AR, Lamb HK; Medline: 96048023 The molecular biology of multidomain proteins. Selected examples." *Eur J Biochem* 1995;232:7-18.

The 3-dehydroquinate synthase EC:4.6.1.3 domain is present in isolation in various bacterial 3-dehydroquinate synthases and also present as a domain in the pentafunctional AROM polypeptide Swiss:P07547 [2]. 3-dehydroquinate (DHQ) synthase catalyses the formation of dehydroquinate (DHQ) and orthophosphate from 3-deoxy-D-arabino heptulosonic 7 phosphate [1]. This reaction is part of the shikimate pathway which is involved in the biosynthesis of aromatic amino acids.

Number of members: 25

- 15 933. Dihydrofolate reductase signature (DiHfolate_red)

Dihydrofolate reductases (EC 1.5.1.3) [1] are ubiquitous enzymes which catalyze the reduction of folic acid into tetrahydrofolic acid. They can be inhibited by a number of antagonists such as trimethoprim and methotrexate which are used as antibacterial or anticancerous agents. A signature pattern was derived from a region in the N-terminal part of these enzymes, which includes a conserved Pro-Trp dipeptide; the tryptophan has been shown [2] to be involved in the binding of substrate by the enzyme.

- Consensus pattern[LVAGC]-[LIF]-G-x(4)-[LIVMF]-P-W-x(4,5)-[DE]-x(3)-[FYIV]-x(3)-[STIQ] Sequences known to belong to this class detected by the patternALL, except for type II bacterial, plasmid-encoded, dihydrofolate reductases which do not belong to the same class of enzymes.

- [1] Harpers' Review of Biochemistry, Lange, Los Altos (1985).
[2] Bolin J.T., Filman D.J., Matthews D.A., Hamlin R.C., Kraut J. J. Biol. Chem. 257:13650-13662(1982).

934. (DIL)

[1] Ponting CP; Medline: 95397417 AF-6/cno: neither a kinesin nor a myosin, but a bit of both." Trends Biochem Sci 1995;20:265-266.

Number of members: 31

935. (DNA_gyraseB_C)

DNA topoisomerase II signature (cross-reference = TOPOISOMERASE_II)

DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type II topoisomerases are ATP-dependent and act by passing a DNA segment through a transient double-strand break. Topoisomerase II is found in phages, archaeobacteria, prokaryotes, eukaryotes, and in African Swine Fever virus (ASF). In bacteriophage T4 topoisomerase II consists of three subunits (the product of genes 39, 52 and 60). In prokaryotes and in archaeobacteria the enzyme, known as DNA gyrase, consists of two subunits (genes *gyrA* and *gyrB* [E2]). In some bacteria, a second type II topoisomerase has been identified; it is known as topoisomerase IV and is required for chromosome segregation, it also consists of two subunits (genes *parC* and *parE*). In eukaryotes, type II topoisomerase is a homodimer.

There are many regions of sequence homology between the different subtypes of topoisomerase II. The relation between the different subunits is shown in the following representation:

<-----About-1400-residues----->

[-----Protein 39.*-----][-----Protein 52-----] Phage T4

[-----gyrB-----*-----][-----gyrA-----] Prokaryote II
Archaeobacteria

[-----parE-----*-----][-----parD-----] Prokaryote IV

[-----*-----] Eukaryote and ASF

*: Position of the pattern.

As a signature pattern for this family of proteins, a region was selected that contains a highly conserved pentapeptide. The pattern is located in *gyrB*, in *parE*, and in protein 39 of phage T4 topoisomerase.

Consensus pattern [LIVMA]-x-E-G-[DN]-S-A-x-[STAG] Sequences known to belong to this class detected by the pattern ALL.

- 5 [1] Sternglanz R. Curr. Opin. Cell Biol. 1:533-535(1990).
 [2] Bjornsti M.-A. Curr. Opin. Struct. Biol. 1:99-103(1991).
 [3] Sharma A., Mondragon A. Curr. Opin. Struct. Biol. 5:39-47(1995).
 [4] Roca J. Trends Biochem. Sci. 20:156-160(1995).
- 10 936. (DNA_topoisolIV)
 DNA topoisomerase II signature (cross-reference = TOPOISOMERASE_II)

- DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type II topoisomerases are ATP-dependent and act by passing a DNA segment through a transient double-strand break.
- 15 Topoisomerase II is found in phages, archaeobacteria, prokaryotes, eukaryotes, and in African Swine Fever virus (ASF). In bacteriophage T4 topoisomerase II consists of three subunits (the product of genes 39, 52 and 60). In prokaryotes and in archaeobacteria the enzyme, known as DNA gyrase, consists of two subunits (genes *gyrA* and *gyrB* [E2]). In some bacteria, a
- 20 second type II topoisomerase has been identified; it is known as topoisomerase IV and is required for chromosome segregation, it also consists of two subunits (genes *parC* and *parE*). In eukaryotes, type II topoisomerase is a homodimer.

- There are many regions of sequence homology between the different subtypes of
- 25 topoisomerase II. The relation between the different subunits is shown in the following representation:

- <-----About-1400-residues----->
- [-----Protein 39.*-----][-----Protein 52-----] Phage T4
- 30 [-----gyrB-----*-----][-----gyrA-----] Prokaryote II Archaeobacteria
- [-----parE-----*-----][-----parD-----] Prokaryote IV
- [-----*-----] Eukaryote and ASF

¹⁸¹: Position of the pattern.

As a signature pattern for this family of proteins, a region was selected that contains a highly conserved pentapeptide. The pattern is located in gyrB, in parE, and in protein 39 of phage T4 topoisomerase.

- 5 Consensus pattern [LIVMA]-x-E-G-[DN]-S-A-x-[STAG] Sequences known to belong to this class detected by the patternALL.

[1] Sternglanz R. Curr. Opin. Cell Biol. 1:533-535(1990).

[2] Bjornsti M.-A. Curr. Opin. Struct. Biol. 1:99-103(1991).

- 10 [3] Sharma A., Mondragon A. Curr. Opin. Struct. Biol. 5:39-47(1995).

[4] Roca J. Trends Biochem. Sci. 20:156-160(1995).

937. Prolyl oligopeptidase family serine active site (DPPIV_N_term)

- 15 The prolyl oligopeptidase family [1,2,3] consist of a number of evolutionary related peptidases whose catalytic activity seems to be provided by a charge relay system similar to that of the trypsin family of serine proteases, but which evolved by independent convergent evolution. The known members of this family are listed below.
- Prolyl endopeptidase (EC 3.4.21.26) (PE) (also called post-proline cleaving enzyme). PE is
- 20 an enzyme that cleaves peptide bonds on the C-terminal side of prolyl residues. The sequence of PE has been obtained from a mammalian species (pig) and from bacteria (*Flavobacterium meningosepticum* and *Aeromonas hydrophila*); there is a high degree of sequence conservation between these sequences.
- *Escherichia coli* protease II (EC 3.4.21.83) (oligopeptidase B) (gene prtB) which cleaves
- 25 peptide bonds on the C-terminal side of lysyl and argininy residues.
- Dipeptidyl peptidase IV (EC 3.4.14.5) (DPP IV). DPP IV is an enzyme that removes N-terminal dipeptides sequentially from polypeptides having unsubstituted N-termini provided that the penultimate residue is proline.
 - Yeast vacuolar dipeptidyl aminopeptidase A (DPAP A) (gene: STE13) which is responsible
- 30 for the proteolytic maturation of the alpha-factor precursor.
- Yeast vacuolar dipeptidyl aminopeptidase B (DPAP B) (gene: DAP2).
 - Acylamino-acid-releasing enzyme (EC 3.4.19.1) (acyl-peptide hydrolase). This enzyme catalyzes the hydrolysis of the amino-terminal peptide bond of an N-acetylated protein to generate a N-acetylated amino acid and a protein with a free amino-terminus.

A conserved serine residue has experimentally been shown (in E.coli protease II as well as in pgi and bacterial PE) to be necessary for the catalytic mechanism. This serine, which is part of the catalytic triad (Ser, His, Asp), is generally located about 150 residues away from the C-terminal extremity of these enzymes (which are all proteins that contains about 700 to 800 amino acids).

Consensus pattern D-x(3)-A-x(3)-[LIVMFYW]-x(14)-G-x-S-x-G-G-[LIVMFYW](2) [S is the active site residue] Sequences known to belong to this class detected by the pattern ALL, except for yeast DPAP A.

Note: these proteins belong to families S9A/S9B/S9C in the classification of peptidases [4,E1].

- [1] Rawlings N.D., Polgar L., Barrett A.J. Biochem. J. 279:907-911(1991).
- [2] Barrett A.J., Rawlings N.D. Biol. Chem. Hoppe-Seyler 373:353-360(1992).
- [3] Polgar L., Szabo E. Biol. Chem. Hoppe-Seyler 373:361-366(1992).
- [4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

938. Deoxyhypusine synthase (DS)

Eukaryotic initiation factor 5A (eIF-5A) contains an unusual amino acid, hypusine [N epsilon-(4-aminobutyl-2-hydroxy)lysine]. The first step in the post-translational formation of hypusine is catalysed by the enzyme deoxyhypusine synthase (DS) EC:1.1.1.249. The modified version of eIF-5A, and DS, are required for eukaryotic cell proliferation [1].

Number of members: 9

- [1] Liao DI, Wolff EC, Park MH, Davies DR; Medline: 98154315 Crystal structure of the NAD complex of human deoxyhypusine synthase: an enzyme with a ball-and-chain mechanism for blocking the active site." Structure 1998;6:23-32.

939. (DUF21)

Many of the sequences in this family are annotated as hemolysins, however this is due to a similarity to Swiss:Q54318 that does not contain this domain. This domain is found in the N-terminus of the proteins adjacent to two intracellular CBS domains CBS.

Number of members: 42

940. (DUF59)

This family includes prokaryotic proteins of unknown function. The family also includes PhaH Swiss:O84984 from *Pseudomonas putida*. PhaH forms a complex with PhaF Swiss:O84982, PhaG Swiss:O84983 and PhaI Swiss:O84985, which hydroxylates phenylacetic acid to 2-hydroxyphenylacetic acid [1]. So members of this family may all be components of ring hydroxylating complexes.

Number of members: 15

[1] Olivera ER, Minambres B, Garcia B, Muniz C, Moreno MA, Ferrandez A, Diaz E, Garcia JL, Luengo JM; Medline: 98263372 Molecular characterization of the phenylacetic acid catabolic pathway in *Pseudomonas putida* U: the phenylacetyl-CoA catabolon." Proc Natl Acad Sci U S A 1998;95:6419-6424.

941. (DUF82)

The protein contains four conserved cysteines that may be involved in metal binding or disulphide bridges.

Number of members: 4

942. Riboflavin kinase / FAD synthetase (FAD_Synth)

This family consists part of the bifunctional enzyme riboflavin kinase / FAD synthetase. These enzymes have both ATP:riboflavin 5'-phospho transferase and ATP:FMN-adenylyltransferase activities [1]. They catalyse the 5'-phosphorylation of riboflavin to FMN and the adenylylation of FMN to FAD [1].

CAUTION: It is not clear if this region of the enzymes catalyses either or both of the enzymatic reactions.

Number of members: 27

- 5 [1] Manstein DJ, Pai EF; Medline: 87057286 Purification and characterization of FAD synthetase from *Brevibacterium ammoniagenes*." J Biol Chem 1986;261:16169-16173.

943. [2Fe-2S] binding domain (fer2_2)

- 10 [1] Romao MJ, Archer M, Moura I, Moura JJ, LeGall J, Engh R, Schneider M, Hof P, Huber R; Medline: 96072968 Crystal structure of the xanthine oxidase-related aldehyde oxidoreductase from *D. gigas*." Science 1995;270:1170-1176.

Number of members: 53

- 15 944. Filovirus glycoprotein (Filo_glycop)

This family includes an extracellular region from the envelope glycoprotein of Ebola and Marburg viruses. This region is also produced as a separate transcript that gives rise to a non-structural, secreted glycoprotein, which is produced in large amounts and has an unknown

- 20 function [1]. Processing of this protein may be involved in viral pathogenicity [2].

Number of members: 23

- [1] Volchkov VE, Feldmann H, Volchkova VA, Klenk HD; Medline: 98245155 Processing of the Ebola virus glycoprotein by the proprotein convertase furin." Proc Natl Acad Sci U S A 1998;95:5762-5767.

[2] Sanchez A, Trappier SG, Mahy BW, Peters CJ, Nichol ST; Medline: 96195018 The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing." Proc Natl Acad Sci U S A 1996;93:3602-3607.

- 30 945. Frataxin-like domain (Frataxin_Cyay)

This family contains proteins that have a domain related to the globular C-terminus of Frataxin the protein that is mutated in Friedreich's ataxia. This domain is found in a family of bacterial proteins. The function of this domain is currently unknown.

Number of members: 12

[1] Gibson TJ, Koonin EV, Musco G, Pastore A, Bork P; Medline: 97084946 "Friedreich's ataxia protein: phylogenetic evidence for mitochondrial dysfunction." Trends Neurosci 1996;19:465-468.

946. (GAF)

Domain present in phytochromes and cGMP-specific phosphodiesterases.

10 Number of members: 296

[1] Aravind L, Ponting CP; Medline: 98094688 "The GAF domain: an evolutionary link between diverse phototransducing proteins." Trends Biochem Sci 1997;22:458-459.

15 947. Galaptin signature (Gal-bind_lectin)

All vertebrates synthesize soluble galactoside-binding lectins [1,2,3] (also known as galectins, galaptins or S-lectin). These carbohydrate-binding proteins are developmentally regulated. Although their exact physiological role is not yet clear they seem to be involved in differentiation, cellular regulation and tissue construction. The sequence of galactoside-binding lectins from electric eel (electrolectin), conger eel (congerin), chicken and a number of mammalian species is known. These lectins are proteins of about 130 to 140 amino acid residues (14 Kd to 16 Kd).

25 A number of other proteins are known to belong to this family:

- Galectin-3 (also known as MAC-2 antigen; CBP-35 or IgE-binding protein), a 35 Kd lectin which binds immunoglobulin E and which is composed of two domains: a N-terminal domain that consist of tandem repeats of a glycine/ proline-rich sequence and a C-terminal galaptin domain.

30 - Galectin-4 [4], which is composed of two galaptin domains.

- Galectin-5.

- Galectin-7 [5], a keratinocyte protein which could be involved in cell-cell and/or cell-matrix interactions necessary for normal growth control.

- Galectin-8 [6], which is composed of two galaptin domains.

- Galectin-9 [7], which is composed of two galactin domains.

- Human eosinophil lysophospholipase (EC 3.1.1.5) [8] (Charcot-Leyden crystal protein), a protein that may have both an enzymatic and a lectin activities. It forms hexagonal bipyramidal crystals in tissues and secretions from sites of eosinophil-associated

5 inflammation.

- Caenorhabditis elegans 32 Kd lactose-binding lectin [9]. This lectin is composed of two galactin domains.

- Caenorhabditis elegans lec-7 and lec-8.

One of the conserved regions of these lectins contains a tryptophan that has been shown [10]
10 to be essential to the binding of galactosides. This region was used as a signature pattern for these proteins.

Consensus pattern W-[GEK]-x-[EQ]-x-[KRE]-x(3,6)-[PCTF]-[LIVMF]-[NQE GSKV]-x-
[GH]-x(3)-[DENKHS]-[LIVMFC] [W binds carbohydrate] Sequences known to belong to
15 this class detected by the pattern ALL_x except for pig galectin 4.

[1] Barondes S.H., Gitt M.A., Leffler H., Cooper D.N.W. Biochimie 70:1627-1632(1988).

[2] Hirabayashi J., Kasai K.-I. J. Biochem. 104:1-4(1988).

[3] Barondes S.H., Castronovo V., Cooper D.N.W., Cummings R.D., Drickamer K., Feizi
20 T., Gitt M.A., Hirabayashi J., Hughes C., Kasai K.-I., Leffler H., Liu F.-T., Lotan R.,
Mercurio A.M., Monsigny M., Pillair S., Poirer F., Raz A., Rigby P.W.J., Rini J.M., Wang
J.L. Cell 76:597-598(1994).

[4] Oda Y., Herrmann J., Gitt M., Turck C.W., Burlingame A.L., Barondes S.H., Leffler H.
J. Biol. Chem. 268:5929-5939(1993).

25 [5] Madsen P., Rasmussen H.H., Flint T., Gromov P., Kruse T.A., Honore B., Vorum H.,
Celis J.E. J. Biol. Chem. 270:5823-5829(1995).

[6] Hadari Y.R., Paz K., Dekel R., Mestrovic T., Accili D., Zick Y. J. Biol. Chem. 270:3447-
3453(1995).

[7] Wada J., Kanwar Y.S. J. Biol. Chem. 272:6078-6086(1997).

30 [8] Ackerman S.J., Corrette S.E., Rosenberg H.F., Bennett J.C., Mastrianni D.M.,
Nicholson-Weller A., Weller P.F., Chin D.T., Tenen D.G. J. Immunol. 150:456-468(1993).

[9] Hirabayashi J., Satoh M., Kasai K.-I. J. Biol. Chem. 267:15485-15490(1992).

[10] Abbott W.M., Feizi T. J. Biol. Chem. 266:5552-5557(1991).

948. (GARS) Phosphoribosylglycinamide synthetase signature (phosphoribosylamine glycine ligase)

PROSITE: PDOC00164; cross-reference(s): PS00184

- 5 [1] catalyzes the second step in the *de novo* biosynthesis of purine, the ATP-dependent addition of 5-phosphoribosylamine to glycine to form 5'phosphoribosylglycinamide.

In bacteria GARS is a monofunctional enzyme (encoded by the *purD* gene), in of a bifunctional enzyme (encoded by the *ADE5,7* gene), in higher eukaryotes it is part, with AIRS and with phosphoribosylglycinamide formyltransferase (GART) of a trifunctional enzyme (GARS-AIRS-GART).

10 enzyme (GARS-AIRS-GART).

The sequence of GARS is well conserved. A highly conserved octapeptide was selected as a signature pattern.

Consensus pattern R-F-G-D-P-E-x-[QM]

- 15 Sequences known to belong to this class detected by the pattern ALL.

[1] Aiba A., Mizobuchi K. J. Biol. Chem. 264:21239-21246(1989).

949. GLTT - GLTT repeat (12 copies)

- 20 This short repeat of unknown function is found in multiple copies in several *C. elegans* proteins. The repeat is five residues long and consists of XGLTT where X can be any amino acid. Number of members: 34.

950. Glu_synthase - Conserved region in glutamate synthase

- 25 This family represents a region of the glutamate synthase protein. This region is expressed as a separate subunit in the glutamate synthase alpha subunit from archaeobacteria, or part of a large multidomain enzyme in other organisms. The aligned region of these proteins contains a putative FMN binding site and Fe-S cluster. Number of members: 44.

- 30 [1] Medline: 97082505. Sequence of the GLT1 gene from *Saccharomyces cerevisiae* reveals the domain structure of yeast glutamate synthase. Filetici P, Martegani MP, Valenzuela L, Gonzalez A, Ballario P; Yeast 1996;12:1359-1366.

951. (Glyco_hydro_2) Glycosyl hydrolases family 2 signatures

GLYCOSYL_HYDROL_F2_1; PS00608; GLYCOSYL_HYDROL_F2_2

It has been shown [1,2,E1] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

-Beta-galactosidases (EC 3.2.1.23) from bacteria such as *Escherichia coli* (genes *lacZ* and *ebgA*), *Clostridium acetobutylicum*, *Clostridium thermosulfurogenes*, *Klebsiella pneumoniae*, *Lactobacillus delbrueckii*, or *Streptococcus thermophilus* and from the fungi *Kluyveromyces lactis*.

-Beta-glucuronidase (EC 3.2.1.31) from *Escherichia coli* (gene *uidA*) and from mammals.

One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [3], in *Escherichia coli lacZ*, to be the general acid/base catalyst in the active site of the enzyme. This region has been used as a signature pattern. A highly conserved region located some sixty residues upstream from the active site glutamate has been selected as a second signature pattern.

Consensus pattern N-x-[LIVMFYWD]-R-[STACN](2)-H-Y-P-x(4)-[LIVMFYWS](2)-x(3)-[DN]-x(2)-G-[LIVMFYW](4) Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [DENQLF]-[KRVW]-N-[HRY]-[STAPPV]-[SAC]-[LIVMFS](3)-W-[GS]-x(2,3)-N-E [E is the active site residue] Sequences known to belong to this class detected by the pattern ALL, except for *Rhizobium meliloti lacZ*.

[1]Henrissat B. *Biochem. J.* 280:309-316(1991).

[2]Schroeder C.J., Robert C., Lenzen G., McKay L.L., Mercenier A. *J. Gen. Microbiol.* 137:369-380(1991).

[3]Gebler J.C., Aebersold R., Withers S.G. *J. Biol. Chem.* 267:11126-11130(1992).

952. (Glyco_hydro_3) Glycosyl hydrolases family 3 active site

PROSITE: PDOC00621. PROSITE cross-reference(s)PS00775; GLYCOSYL_HYDROL_F3

It has been shown [1,2] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

-Beta glucosidases (EC 3.2.1.21) from the fungi *Aspergillus wentii* (A-3), *Hansenula anomala*, *Kluyveromyces fragilis*, *Saccharomycopsis fibuligera*, (BGL1 and BGL2), *Schizophyllum commune* and *Trichoderma reesei* (BGL1).

-Beta glucosidases from the bacteria *Agrobacterium tumefaciens* (Cbg1), *Butyrivibrio fibrisolvens* (bglA), *Clostridium thermocellum* (bglB), *Escherichia coli* (bglX), *Erwinia chrysanthemi* (bgxA) and *Ruminococcus albus*.

-*Alteromonas* strain O-7 beta-hexosaminidase A (EC 3.2.1.52).

5 -*Bacillus subtilis* hypothetical protein yzbA.

-*Escherichia coli* hypothetical protein ycfO and HI0959, the corresponding *Haemophilus influenzae* protein.

One of the conserved regions in these enzymes is centered on a conserved aspartic acid residue which has been shown [3], in *Aspergillus wentii* beta-glucosidase A3, to be
10 implicated in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern[LIVM](2)-[KR]-x-[EOK]-x(4)-G-[LIVMFT]-[LIVT]-[LIVMF]-[ST]-D-
x(2)-[SGADNI] [D is the active site residue]

Sequences known to belong to this class detected by the patternALL.

15

[1]Henrissat B. *Biochem. J.* 280:309-316(1991).

[2]Castle L.A., Smith K.D., Morris R.O. *J. Bacteriol.* 174:1478-1486(1992).

[3]Bause E., Legler G. *Biochim. Biophys. Acta* 626:459-465(1980).

20

953. GP120 - Envelope glycoprotein GP120

The entry of HIV requires interaction of viral GP120 with Swiss:P01730 and a chemokine receptor on the cell surface. Number of members: 17891

25

[1]Medline: 98303379. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA; *Nature* 1998;393:648-659.

954. (GSPII_E) Bacterial type II secretion system protein E signature

PROSITE: PDOC00567. PROSITE cross-reference(s) PS00662; T2SP_E

30

A number of bacterial proteins, some of which are involved in a general secretion pathway (GSP) for the export of proteins (also called the type II pathway) [1,2], have been found to be evolutionary related. These proteins are listed below:

-The 'E' protein from the GSP operon of: *Aeromonas* (gene *exeE*); *Erwinia* (gene *outE*); *Escherichia coli* (gene *yheG*); *Klebsiella pneumoniae* (gene *pulE*); *Pseudomonas aeruginosa* (gene *xcpR*); *Vibrio cholerae* (gene *epsE*) and *Xanthomonas campestris* (gene *xpsE*).

-*Agrobacterium tumefaciens* Ti plasmid *virB* operon protein 11. This protein is required for the transfer of T-DNA to plants.

-*Bacillus subtilis* *comG* operon protein 1 which is required for the uptake of DNA by competent *Bacillus subtilis* cells.

-*Aeromonas hydrophila* *tapB*, involved in type IV pilus assembly.

-*Pseudomonas* protein *pilB*, which is essential for the formation of the pili.

-*Pseudomonas aeruginosa* protein twitching mobility protein *pilT*.

-*Neisseria gonorrhoeae* type IV pilus assembly protein *pilF*.

-*Vibrio cholerae* protein *tcpT*, which is involved in the biosynthesis of the *tcp* pilus.

-*Escherichia coli* protein *hofB* (*hopB*).

-*Escherichia coli* hypothetical protein *ycgB*.

-*Escherichia coli* hypothetical protein *yggR*.

These proteins have from 344 (*pilT* and *virB11*) to 568 (*tapB*) amino acids, they are probably cytoplasmically located and, on the basis of the presence of a conserved P-loop region (see <PDOC00017>), probably bind ATP. A region that overlaps the 'B' motif of ATP-binding proteins was selected as a signature pattern.

Consensus pattern[LIVM]-R-x(2)-P-D-x-[LIVM](3)-G-E-[LIVM]-R-D

Sequences known to belong to this class detected by the patternALL, except for *ycgB*.

[1]Salmond G.P.C., Reeves P.J. Trends Biochem. Sci. 18:7-12(1993).

[2]Hobbs M., Mattick J.S. Mol. Microbiol. 10:233-243(1993).

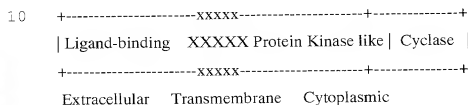
955. (guanylate_cyc) Guanylate cyclases signature

PROSITE: PDOC00425. PROSITE cross-reference(s) PS00452;

GUANYLATE_CYCLASES Guanylate cyclases (EC 4.6.1.2) [1 to 4] catalyze the formation of cyclic GMP (cGMP) from GTP. cGMP acts as an intracellular messenger, activating cGMP dependent kinases and regulating CGMP-sensitive ion channels. The role of cGMP as a second messenger in vascular smooth muscle relaxation and retinal photo-transduction is well established. Guanylate cyclase is found both in the soluble and particular

fraction of eukaryotic cells. The soluble and plasma membrane-bound forms differ in structure, regulation and other properties.

Most currently known plasma membrane-bound forms are receptors for small polypeptides. The topology of such proteins is the following: they have a N-terminal extracellular domain which acts as the ligand binding region, then a transmembrane domain, followed by a large cytoplasmic C-terminal region that can be subdivided into two domains: a protein kinase-like domain that appears important for proper signalling and a cyclase catalytic domain. This topology is schematically represented below.



15 The known guanylate cyclase receptors are:

- The sea-urchins receptors for speract and resact, which are small peptides that stimulate sperm motility and metabolism.
- The receptors for natriuretic peptides (ANF). Two forms of ANF receptors with guanylate cyclase activity are currently known: GC-A (or ANP-A) which seems specific to atrial natriuretic peptide (ANP), and GC-B (or ANP-B) which seems to be stimulated more effectively by brain natriuretic peptide (BNP) than by ANP.
- The receptor for Escherichia coli heat-stable enterotoxin (GC-C). The endogenous ligand for this intestinal receptor seems to be a small peptide called guanylin.
- Retinal guanylate cyclase (retGC) which probably plays a specific functional role in the rods and/or cones of photoreceptors. It is not known if this protein acts as receptor, but its structure is similar to that of the other plasma membrane-bound GCs.

The soluble forms of guanylate cyclase are cytoplasmic heterodimers. The two subunits, alpha and beta are proteins of from 70 to 82 Kd which are highly related. Two forms of beta subunits are currently known: beta-1 which seems to be expressed in lung and brain, and beta-2 which is more abundant in kidney and liver.

The membrane and cytoplasmic forms of guanylate cyclase share a conserved domain which is probably important for the catalytic activity of the enzyme. Such a domain is also found twice in the different forms of membrane-bound adenylate cyclases (also known as

class-III) [5,6] from mammals, slime mold or *Drosophila*. A consensus pattern was derived from the most conserved region in that domain.

Consensus pattern G-V-[LIVM]-x(0,1)-G-x(5)-[FY]-x-[LIVM]-[FYW]-[GS]-[DNTHKW]-
5 [DNT]-[IV]-[DNTA]-x(5)-[DE]

Sequences known to belong to this class detected by the pattern ALL, except for the sea urchin *Arbacia punctulata* resact receptor which lack this domain.

Note this pattern will detect both domains of adenylate cyclases class-III.

- 10 [1]Koesling D., Boehme E., Schultz G. FASEB J. 5:2785-2791(1991).
- [2]Garbers D.L. New Biol. 2:499-504(1990).
- [3]Garbers D.L. Cell 71:1-4(1992).
- [4]Yuen P.S.T., Garbers D.L. Annu. Rev. Neurosci. 15:193-225(1992).
- [5]Iyengar R. FASEB J. 7:768-775(1993).
- 15 [6]Barzu O., Danchin A. Prog. Nucleic Acid Res. Mol. Biol. 49:241-283(1994).

956. Hemolysin-type calcium-binding region signature (HemolysinCabinD)

- Gram-negative bacteria produce a number of proteins which are secreted into the growth medium by a mechanism that does not require a cleaved N-terminal signal sequence. These proteins, while having different functions, seem [1] to share two properties: they bind calcium and they contain a variable number of tandem repeats consisting of a nine amino acid motif rich in glycine, aspartic acid and asparagine. It has been shown [2] that such a domain is involved in the binding of calcium ions in a parallel beta roll structure. The proteins which
- 25 are currently known to belong to this category are:
 - Hemolysins from various species of bacteria. Bacterial hemolysins are exotoxins that attack blood cell membranes and cause cell rupture. The hemolysins which are known to contain such a domain are those from: *E. coli* (gene hlyA), *A. pleuropneumoniae* (gene appA), *A. actinomycetemcomitans* and *P. haemolytica* (leukotoxin) (gene lktA).
 - 30 - Cyclolysin from *Bordetella pertussis* (gene cyaA). A multifunctional protein which is both an adenylate cyclase and a hemolysin.
 - Extracellular zinc proteases: serralsin (EC 3.4.24.40) from *Serratia*, prtB and prtC from *Erwinia chrysanthemi* and aprA from *Pseudomonas aeruginosa*.
 - Nodulation protein nodO from *Rhizobium leguminosarum*.

A signature pattern was derived from conserved positions in the sequence of the calcium-binding domain.

- Consensus pattern D-x-[LI]-x(4)-G-x-D-x-[LI]-x-G-G-x(3)-D Sequences known to belong to
5 this class detected by the pattern ALL.

Note: This pattern is found once in nodO and the extracellular proteases but up to 5 times in some hemolysin/cyclolysins.

- 10 [1] Economou A., Hamilton W.D.O., Johnston A.W.B., Downie J.A. EMBO J. 9:349-354(1990).

[2] Baumann U., Wu S., Flaherty K.M., McKay D.B. EMBO J. 12:3357-3364(1993).

957. Hint module (Hint)

- 15 This is an alignment of the Hint module in the Hedgehog proteins. It does not include any Inteins which also possess the Hint module.

Number of members: 36

- 20 [1] Hall TM, Porter JA, Young KE, Koonin EV, Beachy PA, Leahy DJ; Medline: 97474313
Crystal structure of a Hedgehog autoprocessing domain: homology between Hedgehog and self-splicing proteins." Cell 1997;91:85-97.

958. Hydantoinase/oxoprolinase (Hydantoinase)

- 25 This family includes the enzymes hydantoinase and oxoprolinase EC:3.5.2.9. Both reactions involve the hydrolysis of 5-membered rings via hydrolysis of their internal imide bonds [1].
Number of members: 14

- 30 [1] Ye GJ, Breslow EB, Meister A, Guo-jie GE\$[corrected to Ye GJ]; Medline: 97113037
The amino acid sequence of rat kidney 5-oxo-L-prolinase determined by cDNA cloning"
[published erratum appears in J Biol Chem 1997 Feb 14;272(7):4646] J Biol Chem
1996;271:32293-32300.

959. IMP dehydrogenase / GMP reductase signature (IMPDH_N)

IMP dehydrogenase (EC 1.1.1.205) (IMPDH) catalyzes the rate-limiting reaction of de novo GTP biosynthesis, the NAD-dependent reduction of IMP into XMP [1]. Inhibition of IMP dehydrogenase activity results in the cessation of DNA synthesis. As IMP dehydrogenase is associated with cell proliferation, it is a possible target for cancer chemotherapy. Mammalian and bacterial IMPDHs are tetramers of identical chains. There are two IMP dehydrogenase isozymes in humans [2].

GMP reductase (EC 1.6.6.8) catalyzes the irreversible and NADPH-dependent reductive deamination of GMP into IMP [3]. It converts nucleobase, nucleoside and nucleotide derivatives of G to A nucleotides, and maintains intracellular balance of A and G nucleotides.

IMP dehydrogenase and GMP reductase share many regions of sequence similarity. One of these regions is centered on a cysteine residue thought [3] to be involved in binding IMP. This region was used as a signature pattern.

Consensus pattern[LIVM]-[RK]-[LIVM]-G-[LIVM]-G-x-G-S-[LIVM]-C-x-T [C is the putative IMP-binding residue] Sequences known to belong to this class detected by the pattern ALL.

[1] Collart F.R., Huberman E. J. Biol. Chem. 263:15769-15772(1988).

[2] Natsumeda Y., Ohno S., Kawasaki H., Konno Y., Weber G., Suzuki K. J. Biol. Chem. 265:5292-5295(1990).

[3] Andrews S.C., Guest J.R. Biochem. J. 255:35-43(1988).

960. impB/mucB/samB family (IMS)

These proteins are involved in UV protection (Swiss).

Number of members: 38

961. Type II intron maturase (Intron_maturas2)

Group II introns use intron-encoded reverse transcriptase, maturase and DNA endonuclease activities for site-specific insertion into DNA [2]. Although this type of intron is self splicing in vitro they require a maturase protein for splicing in vivo. It has been shown that a specific region of the a12 intron is needed for the maturase function [1]. This region was found to be conserved in group II introns and called domain X [3].

Number of members: 335

[1] Moran JV, Mecklenburg KL, Sass P, Belcher SM, Mahne D, Lewin A, Perlman P; Medline: 94301788 Splicing defective mutants of the COXI gene of yeast mitochondrial DNA: initial definition of the maturase domain of the group II intron a12. Nucleic Acids Res 1994;22:2057-2064.

[2] Guo H, Zimmerly S, Perlman PS, Lambowitz AM; Medline: 98031910 Group II intron endonucleases use both RNA and protein subunits for recognition of specific sequences in double-stranded DNA." EMBO J 1997;16:6835-6848.

[3] Mohr G, Perlman PS, Lambowitz AM; Medline: 94077696 Evolutionary relationships among group II intron-encoded proteins and identification of a conserved domain that may be related to maturase function." Nucleic Acids Res 1993;21:4991-4997.

962. LAGLIDADG endonuclease (Intron_maturase)

[1] Heath PJ, Stephens KM, Monnat RJ Jr, Stoddard BL; Medline: 97331323 The structure of I-Crel, a group I intron-encoded homing endonuclease." Nat Struct Biol 1997;4:468-476.

[2] Belfort M, Roberts RJ; Medline: 97402526 Homing endonucleases: keeping the house in order." Nucleic Acids Res 1997;25:3379-3388.

[3] Dalgaard JZ, Klar AJ, Moser MJ, Holley WR, Chatterjee A, Mian IS; Medline: 98026854 Statistical modeling and analysis of the LAGLIDADG family of site-specific endonucleases and identification of an intein that encodes a site-specific endonuclease of the HNH family." Nucleic Acids Res 1997;25:4626-4638.

Number of members: 220

963. Isopentenyl transferase (IPT)

Isopentenyl transferase / dimethylallyl transferase synthesizes isopentenyladenosine 5'-monophosphate, a cytokinin that induces shoot formation on host plants infected with the Ti plasmid [1].

Number of members: 16

5

[1] Canaday J, Gerad JC, Crouzet P, Otten L; Medline: 93101133 "Organization and functional analysis of three T-DNAs from the vitopine Ti plasmid pTiS4." Mol Gen Genet 1992;235:292-303.

10 964. Laminin EGF-like (Domains III and V) (laminin_EGF)

This family is like EGF but has 8 conserved cysteines instead of 6.

Number of members: 501

15 [1] Engel J; Medline: 93041759 "Laminins and other strange proteins." Biochemistry 1992;31:10643-10651.

965. Legume lectins signatures (lectin_legA)

20 Leguminous plants synthesize sugar-binding proteins which are called legume lectins [1,2]. These lectins are generally found in the seeds. The exact function of legume lectins is not known but they may be involved in the attachment of nitrogen-fixing bacteria to legumes and in the protection against pathogens. Legume lectins bind calcium and manganese (or other transition metals).

25

Legume lectins are synthesized as precursor proteins of about 230 to 260 amino acid residues. Some legume lectins are proteolytically processed to produce two chains: beta (which corresponds to the N-terminal) and alpha (C-terminal). The lectin concanavalin A (conA) from jack bean is exceptional in that the two chains are transposed and ligated (by formation of a new peptide bond). The N-terminus of mature conA thus corresponds to that of the alpha chain and the C-terminus to the beta chain.

30

Two signature patterns were developed specific to legume lectins: the first is located in the C-terminal section of the beta chain and contains a conserved aspartic acid residue important for

the binding of calcium and manganese; the second one is located in the N-terminal of the alpha chain.

Consensus pattern [LIV]-[STAG]-V-[DEQV]-[FLI]-D-[ST] [D binds manganese and calcium] Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [LIV]-x-[EDQ]-[FYWKR]-V-x-[LIVF]-G-[LF]-[ST] Sequences known to belong to this class detected by the pattern ALL.

[1] Sharon N., Lis H. FASEB J. 4:3198-320(1990).

[2] Lis H., Sharon N. Annu. Rev. Biochem. 55:33-37(1986).

966. Malate synthase signature (malate_synthase)

Malate synthase (EC 4.1.3.2) catalyzes the aldol condensation of glyoxylate with acetyl-CoA to form malate - the second step of the glyoxylate bypass, an alternative to the tricarboxylic acid cycle in bacteria, fungi and plants. Malate synthase is a protein of 530 to 570 amino acids whose sequence is highly conserved across species [1]. As a signature pattern, a very conserved region was selected in the central section of the enzyme.

Consensus pattern[KR]-[DENQ]-H-x(2)-G-L-N-x-G-x-W-D-Y-[LIVM]-F Sequences known to belong to this class detected by the pattern ALL.

[1] Bruinenberg P.G., Blaauw M., Kazemier B., Ab G. Yeast 6:245-254(1990).

967. MatK/TrnK amino terminal region (MatK_N)

[1] Mohr G, Perlman PS, Lambowitz AM: Medline: 94077696 Evolutionary relationships among group II intron-encoded proteins and identification of a conserved domain that may be related to maturase function." Nucleic Acids Res 1993;21:4991-4997.

Number of members: 495

968. MOZ/SAS family (MOZ_SAS)

This region of these proteins has been suggested to be homologous to acetyltransferases [1]. However the similarity is not supported by standard sequence analysis.

Number of members: 15

5

[1] Kamine J, Elangovan B, Subramanian T, Coleman D, Chinnadurai G; Medline: 96182937 Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 Tat transactivator." Virology 1996;216:357-366.

10

[2] Reifsnnyder C, Lowell J, Clarke A, Pillus L; Medline: 96376969 Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases" [see comments] [published erratum appears in Nat Genet 1997 May;16(1):109] Nat Genet 1996;14:42-49.

969. mRNA capping enzyme (mRNA_cap_enzyme)

15

[1] Hakansson K, Doherty AJ, Shuman S, Wigley DB; Medline: 97304383 X-ray crystallography reveals a large conformational change during guanyl transfer by mRNA capping enzymes." Cell 1997;89:545-553.

20

Number of members: 7

970. DNA mismatch repair proteins mutS family signature (MutS_C)

25

Mismatch repair contributes to the overall fidelity of DNA replication [1]. It involves the correction of mismatched base pairs that have been missed by the proofreading element of the DNA polymerase complex. The sequence of some proteins involved in mismatch repair in different organisms have been found to be evolutionary related [2,3]. One of these families is called mutS [4,E1], it consists of:

30

- Prokaryotic protein mutS protein (also called hexA in *Streptococcus pneumoniae*). Muts is thought to carry out the mismatch recognition step of DNA repair.
- Eukaryotic MSH1, which is involved in mitochondrial DNA repair.
- Eukaryotic MSH2, which is involved in nuclear postreplication mismatch repair. MSH2 heterodimerizes with MSH6. In man, MSH2 is involved in a form of familial hereditary nonpolyposis colon cancer (HNPCC).

- Eukaryotic MSH3, which is probably involved in the repair of large loops.
- Eukaryotic MSH4, which is involved in meiotic recombination.
- Eukaryotic MSH5, which is involved in meiotic recombination.
- Eukaryotic MSH6 (also known as G/T mismatch binding protein), a DNA-repair protein that binds to G/T mismatches through heterodimerization with MSH2.
- Prokaryotic protein mutS2 whose function is not yet known.
- A coral (*Sarcophyton glaucum*) mitochondrial encoded mutS-like protein.

As a signature pattern for this class of mismatch repair proteins a region rich in glycine and negatively charged residues was selected. This region is found in the C-terminal section of these proteins; about 80 residues to the C-terminal of an ATP-binding site motif 'A' (P-loop) (see <PDOC00017>).

Consensus pattern[ST]-[LIVMF]-x-[LIVM]-x-D-E-[LIVMFY]-[GC]-[RKH]-G-[GST]- x(4)-G Sequences known to belong to this class detected by the pattern ALL, except for mutS2.

- [1] Modrich P. Annu. Rev. Biochem. 56:435-466(1987).
- [2] Haber L.T., Walker G.C. EMBO J. 10:2707-2715(1991).
- [3] New L., Liu K., Crouse G.F. Mol. Gen. Genet. 239:97-108(1993).
- [4] Eisen J.A. Nucleic Acids Res. 26:4291-4300(1998).

971. MutS family, N-terminal putative DNA binding domain (MutS_N)

This family consists of the N-terminal region of proteins in the mutS family of DNA mismatch repair proteins and is found associated with MutS_C located in the C-terminal region. The mutS family of proteins is named after the salmonella typhimurium MutS protein involved in mismatch repair; other members of the family included the eukaryotic MSH 1,2,3,4,5 and 6 proteins. These have various roles in DNA repair and recombination. Human MSH has been implicated in non-polyposis colorectal carcinoma (HNPCC) and is a mismatch binding protein [2]. The aligned region corresponds in part with domains A1, A2 (which may bind DNA) and B (which binds dsDNA in vitro) from *T. thermophilus* MutS as characterised in [1].

Number of members: 43

972. Domain in Myosin and Kinesin Tails (MyTH4)

Domain present twice in myosin-VIIa, and also present in 3 other myosins.

[1] Chen ZY, Hasson T, Kelley PM, Schwender BJ, Schwartz MF, Ramakrishnan M, Kimberling WJ, Mooseker MS, Corey DP; Medline: 97038686 Molecular cloning and domain structure of human myosin-VIIa, the gene product defective in Usher syndrome 1B." Genomics 1996;36:440-448.

Number of members: 21

973. Sodium and potassium ATPases beta subunits signatures (Na_K-ATPase)

The sodium pump (Na⁺,K⁺ ATPase), located in the plasma membrane of all animal cells [1], is an heterotrimer of a catalytic subunit (alpha chain), a glycoprotein subunit of about 34 Kd (beta chain) and a small hydrophobic protein of about 6 Kd. The beta subunit seems [2] to regulate, through the assembly of alpha/beta heterodimers, the number of sodium pumps transported to the plasma membrane.

Structurally the beta subunit is composed of a charged cytoplasmic domain of about 35 residues, followed by a transmembrane region, and a large extracellular domain that contains three disulfide bonds and glycosylation sites. This structure is schematically represented in the figure below.

+---+ +--+ +-----+ |||||

xxxxxxxxxxxxxxxxxxxxxxxxCxxxxCxCxxCxxxxxxx CxxxxxxxxxxCxxxx

***** <-Cyt-><TM><-----Extracellular----->

'C': conserved cysteine involved in a disulfide bond.

'*': position of the patterns.

Two isoforms of the beta subunit (beta-1 and beta-2) are currently known; they share about 50% sequence identity. Gastric (K⁺, H⁺) ATPase (proton pump) responsible for acid production in the stomach consist of two subunits [3]; the beta chain is highly similar to the sodium pump beta subunits. Two signature patterns were developed for beta subunits. The

first is located in the cytoplasmic domain, while the second is found in the extracellular domain and contains two of the cysteines involved in disulfide bonds.

Consensus pattern [FYW]-x(2)-[FYW]-x-[FYW]-[DN]-x(6)-[LIVM]-G-R-T-x(3)-W

5 Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [RK]-x(2)-C-[RKOWI]-x(5)-L-x(2)-C-[SA]-G [The two C's are involved in disulfide bonds] Sequences known to belong to this class detected by the pattern ALL, except for the beta subunit of the sodium pump of brine shrimp whose sequence is highly

10 divergent in that region.

[1] Horisberger J.D., Lemas V., Krahenbul J.P., Rossier B.C. *Annu. Rev. Physiol.* 53:565-584(1991).

[2] McDonough A.A., Gerring K., Farley R.A. *FASEB J.* 4:1598-1605(1990).

15 [3] Toh B.-H., Gleeson P.A., Simpson R.J., Moritz R.L., Callaghan J.M., Goldkorn I., Jones C.M., Martinelli T.M., Mu F.-T., Humphris D.C., Pettitt J.M., Mori Y., Masuda T., Sobieszczuk P., Weinstock J., Mantamadiotis T., Baldwin G.S. *Proc. Natl. Acad. Sci. U.S.A.* 87:6418-6422(1990).

20 974. Respiratory-chain NADH dehydrogenase subunit 1 signatures (NADHdh)

Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there are fifteen which are located in the membrane part, seven of which are encoded by the mitochondrial and chloroplast genomes of most species. The most conserved of these organelle-encoded subunits is known as subunit 1 (gene ND1 in mitochondrion, and NDH1 in chloroplast) and seems to contain the ubiquinone binding site.

30

The ND1 subunit is highly similar to subunit 4 of *Escherichia coli* formate hydrogenlyase (gene hycD), subunit C of hydrogenase-4 (gene hyfC). *Paracoccus denitrificans* NQO8 and *Escherichia coli* nuoH NADH-ubiquinone oxidoreductase subunits also belong to this family [3]. Two signature patterns were developed based on conserved regions of this subunit.

Consensus pattern G-[LIVMFYKRS]-[LIVMAGP]-Q-x-[LIVMFY]-x-D-[AGIM]-[LIVMFTA]-K-[LVMYST]-[LIVMFYG]-x-[KR]-[EQG] Sequences known to belong to this class detected by the pattern ALL, except for watermelon and Leishmania ND1.

5

Consensus pattern P-F-D-[LIVMFYQ]-[STAGPVM]-E-[GAC]-E-x-[EQ]-[LIVMS]-x(2)-G Sequences known to belong to this class detected by the pattern ALL, except for Chlamydomonas reinhardtii and Pisaster ochraceus ND1, and tobacco NDH1.

10 [1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).

[2] Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).

[3] Weidner U., Geier S., Ptöck A., Friedrich T., Leif H., Weiss H. J. Mol. Biol. 233:109-122(1993).

15 975. Nickel-dependent hydrogenases large subunit signatures (NiFeSe_Hases)

Hydrogenases are enzymes that catalyze the reversible activation of hydrogen and which occur widely in prokaryotes as well as in some eukaryotes. There are various types of hydrogenases, but all of them seem to contain at least one iron-sulfur cluster. They can be broadly divided into two groups: hydrogenases containing nickel and, in some cases, also selenium (the [NiFe] and [NiFeSe] hydrogenases) and those lacking nickel (the [Fe] hydrogenases).

The [NiFe] and [NiFeSe] hydrogenases are heterodimer that consist of a small subunit that contains a signal peptide and a large subunit. All the known large subunits seem to be evolutionary related [1]; they contain two Cys-x-x-Cys motifs; one at their N-terminal end; the other at their C-terminal end. These four cysteines are involved in the binding of nickel [2]. In the [NiFeSe] hydrogenases the first cysteine of the C-terminal motif is a selenocysteine which has experimentally been shown to be a nickel ligand [3]. Two patterns were developed which are centered on the Cys-x-x-Cys motifs.

Alcaligenes eutrophus possess a NAD-reducing cytoplasmic hydrogenase (hoxS) [4]; this enzyme is composed of four subunits. Two of these subunits (beta and delta) are responsible for the hydrogenase reaction and are evolutionary related to the large and small subunits of

membrane-bound hydrogenases. The alpha subunit of coenzyme F420 hydrogenase (EC 1.12.99.1) (FRH) from archaeobacterial methanogens also belongs to this family.

Consensus pattern R-G-[LIVMF]-E-x(15)-[QESM]-R-x-C-G-[LIVM]-C [The two C's are nickel ligands] Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [FY]-D-P-C-[LIM]-[ASG]-C-x(2,3)-H [The two C's are nickel ligands] Sequences known to belong to this class detected by the pattern ALL.

- 10 [1] Menon N.K., Robbins J., Peck H.D. Jr., Chatelus C.Y., Choi E.-S., Przybyla A.E. J. Bacteriol. 172:1969-1977(1990).
- [2] Volbeda A., Charon M.-H., Piras C., Hatchikian E.C., Frey M., Fontecilla-Camps J.C. Nature 373:580-587(1995).
- [3] Eidsness M.K., Scott R.A., Prickrill B., der Vartanian D.V., LeGall J., Moura I., Moura J.J.G., Peck H.D. Jr. Proc. Natl. Acad. Sci. U.S.A. 86:147-151(1989).
- 15 [4] Tran-Betcke A., Warnecke U., Boecker C., Zaborosch C., Friedrich B. J. Bacteriol. 172:2920-2929(1990).

976. NADH-Ubiquinone oxidoreductase (complex I), chain 5 C-terminus (oxidored_q1_C)

20

This sub-family represents a carboxyl terminal extension of oxidored_q1. Only NADH-Ubiquinone chain 5 from chloroplasts are in this family. This sub-family is part of complex I which catalyses the transfer of two electrons from NADH to ubiquinone in a reaction that is associated with proton translocation across the membrane.

25 Number of members: 572

[1] Walker JE; Medline: 93110040 The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains." Q Rev Biophys 1992;25:253-324.

30 977. NADH-Ubiquinone oxidoreductase (complex I), chain 5 N-terminus (oxidored_q1_N)

This sub-family represents an amino terminal extension of oxidored_q1. Only NADH-Ubiquinone chain 5 and eubacterial chain L are in this family. This sub-family is part of

complex I which catalyses the transfer of two electrons from NADH to ubiquinone in a reaction that is associated with proton translocation across the membrane.

Number of members: 546

- 5 [1] Walker JE; Medline: 93110040 The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains." Q Rev Biophys 1992;25:253-324.

978. oxidored_q2. NADH-UBIQUINONE OXIDOREDUCTASE CHAIN 4L (EC 1.6.5.3). ND4L OR NAD4L. Arabidopsis thaliana (Mouse-ear cress). Mitochondrion. OC Eukaryota;

- 10 Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; Rosidae; eurosids II; Brassicales; Brassicaceae; Arabidopsis.

CATALYTIC ACTIVITY: NADH + UBIQUINONE = NAD(+) + UBIQUINOL.

[1] SEQUENCE FROM N.A. MEDLINE; 93156682. Brandt P., Sunkel S., Unseld M.,

- 15 Brennicke A., Knoop V.; "The nad4L gene is encoded between exon c of nad5 and orf25 in the Arabidopsis mitochondrial genome."; Mol. Gen. Genet. 236:33-38(1992).

[2] SEQUENCE FROM N.A. STRAIN=CV. COLUMBIA; MEDLINE; 97141919 Unseld M., Marienfeld J.R., Brandt P., Brennicke A.; "The mitochondrial genome of Arabidopsis thaliana contains 57 genes in 366,924 nucleotides."; Nat. Genet. 15:57-61(1997).

20

979. oxidored_q4. Protein name NADH-PLASTOQUINONE OXIDOREDUCTASE CHAIN 3, CHLOROPLAST. Synonym(s)EC 1.6.5.3. Gene name(s)NDHC OR NDH3 From Zea mays (Maize) Encoded on Chloroplast. Taxonomy Eukaryota; Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Zea.

25

CATALYTIC ACTIVITY: NADH + PLASTOQUINONE = NAD(+) + PLASTOQUINOL.

SIMILARITY: BELONGS TO THE COMPLEX I SUBUNIT 3 FAMILY.

[1] SEQUENCE FROM N.A. MEDLINE; 89281491. Steinmueller K., Ley A.C., Steinmetz

30

A.A., Sayre R.T., Bogorad L.; "Characterization of the ndhC-psbG-ORF157/159 operon of maize plastid DNA and of the cyanobacterium Synechocystis sp. PCC6803."; Mol. Gen. Genet. 216:60-69(1989).

[2] SEQUENCE FROM N.A. MEDLINE; 95395841. Maier R.M., Neckermann K., Igloi G.L., Koessel H.; "Complete sequence of the maize chloroplast genome: gene content,

hotspots of divergence and fine tuning of genetic information by transcript editing."; J. Mol. Biol. 251:614-628(1995).

980. PAC: PAC motif

- 5 PAC motif occurs C-terminal to a subset of all known PAS motifs. It is proposed to contribute to the PAS domain fold [3]. Number of members: 181

[1] Medline: 97446881 PAS domain S-boxes in archaea, bacteria and sensors for oxygen and redox. Zhulin IB, Taylor BL, Dixon R; Trends Biochem Sci 1997;22:331-333.

- 10 [2] Medline: 95275818. 1.4 Å structure of photoactive yellow protein, a cytosolic photoreceptor: unusual fold, active site, and chromophore. Borgstahl GE, Williams DR, Getzoff ED; Biochemistry 1995;34:6278-6287.

[3] Medline: 98044337. PAS: a multifunctional domain family comes to light. Ponting CP, Aravind L; Curr Biol 1997;7:674-677.

15

981. PARP: Poly(ADP-ribose) polymerase catalytic region.

Poly(ADP-ribose) polymerase catalyses the covalent attachment of ADP-ribose units from NAD⁺ to itself and to a limited number of other DNA binding proteins, which decreases their affinity for DNA. Poly(ADP-ribose) polymerase is a regulatory component induced by DNA damage.

20

The carboxyl-terminal region is the most highly conserved region of the protein. Experiments have shown that a carboxyl 40 kDa fragment is still catalytically active [2]. Number of members: 19

25

[1] Medline: 96353841 Structure of the catalytic fragment of poly(AD-ribose) polymerase from chicken. Ruf A, Mennissier de Murcia J, de Murcia G, Schulz GE; Proc Natl Acad Sci U S A 1996;93:7481-7485.

- [2] Medline: 93293867 The carboxyl-terminal domain of human poly(ADP-ribose)
30 polymerase. Overproduction in Escherichia coli, large scale purification, and characterization. Simonin F, Hofferer L, Panzeter PL, Muller S, de Murcia G, Althaus FR; J Biol Chem 1993;268:13454-13461.

982. PC_rep: Proteasome/cyclosome repeat

[1] Medline: 97348748 A repetitive sequence in subunits of the 26S proteasome and 20S cyclosome (anaphase-promoting complex). Lupas A, Baumeister W, Hofmann K; Trends Biochem Sci 1997;22:195-196.

Number of members: 112

5

983. Peptidase_M1: Peptidase family M1

Members of this family are aminopeptidases. The members differ widely in specificity, hydrolysing acidic, basic or neutral N-terminal residues. This family includes leukotriene-A4 hydrolase Swiss:P09960, this enzyme also has an aminopeptidase activity [1].

10 members: 72

[1] Medline: 95405261 Evolutionary families of metallopeptidases. Rawlings ND, Barrett AJ; Meth Enzymol 1995;248:183-228.

15 984. Neutral zinc metallopeptidases, zinc-binding region signature (Peptidase_M8) PROSITE cross-reference(s) PS00142; ZINC_PROTEASE

The majority of zinc-dependent metallopeptidases (with the notable exception of the carboxypeptidases) share a common pattern of primary structure [1,2,3] in the part of their sequence involved in the binding of zinc, and can be grouped together as a superfamily, known as the metzincins, on the basis of this sequence similarity. They can be classified into a number of distinct families [4,E1] which are listed below along with the proteases which are currently known to belong to these families.

Family M1

- 25 - Bacterial aminopeptidase N (EC 3.4.11.2) (gene pepN).
 - Mammalian aminopeptidase N (EC 3.4.11.2).
 - Mammalian glutamyl aminopeptidase (EC 3.4.11.7) (aminopeptidase A). It may play a role in regulating growth and differentiation of early B-lineage cells.
 - Yeast aminopeptidase yscII (gene APE2).
 30 - Yeast alanine/arginine aminopeptidase (gene AAP1).
 - Yeast hypothetical protein YIL137c.
 - Leukotriene A-4 hydrolase (EC 3.3.2.6). This enzyme is responsible for the hydrolysis of an epoxide moiety of LTA-4 to form LTB-4; it has been shown that it binds zinc and is capable of peptidase activity.

Family M2

- Angiotensin-converting enzyme (EC 3.4.15.1) (dipeptidyl carboxypeptidase I) (ACE) the enzyme responsible for hydrolyzing angiotensin I to angiotensin II. There are two forms of ACE: a testis-specific isozyme and a somatic isozyme which has two active centers.

5 Family M3

- Thimet oligopeptidase (EC 3.4.24.15), a mammalian enzyme involved in the cytoplasmic degradation of small peptides.

- Neurolysin (EC 3.4.24.16) (also known as mitochondrial oligopeptidase M or microsomal endopeptidase).

10 - Mitochondrial intermediate peptidase precursor (EC 3.4.24.59) (MIP). It is involved the second stage of processing of some proteins imported in the mitochondrion.

- Yeast saccharolysin (EC 3.4.24.37) (proteinase yscD).

- Escherichia coli and related bacteria dipeptidyl carboxypeptidase (EC 3.4.15.5) (gene dcp).

15 - Escherichia coli and related bacteria oligopeptidase A (EC 3.4.24.70) (gene opdA or prlC).

- Yeast hypothetical protein YKL134c.

Family M4

- Thermostable thermolysins (EC 3.4.24.27), and related thermolabile neutral proteases (bacillolysins) (EC 3.4.24.28) from various species of Bacillus.

20 - Pseudolysin (EC 3.4.24.26) from Pseudomonas aeruginosa (gene lasB).

- Extracellular elastase from Staphylococcus epidermidis.

- Extracellular protease prt1 from Erwinia carotovora.

- Extracellular minor protease smp from Serratia marcescens.

- Vibriolysin (EC 3.4.24.25) from various species of Vibrio.

25 - Protease prtA from Listeria monocytogenes.

- Extracellular proteinase proA from Legionella pneumophila.

Family M5

- Mycolysin (EC 3.4.24.31) from Streptomyces cacaoi.

30

Family M6

- Immune inhibitor A from Bacillus thuringiensis (gene ina). Ina degrades two classes of insect antibacterial proteins, attacins and cecropins.

Family M7

- *Streptomyces* extracellular small neutral proteases

Family M8

- 5 - Leishmanolysin (EC 3.4.24.36) (surface glycoprotein gp63), a cell surface protease from various species of *Leishmania*.

Family M9

- 10 - Microbial collagenase (EC 3.4.24.3) from *Clostridium perfringens* and *Vibrio alginolyticus*.

Family M10A

- Serralyisin (EC 3.4.24.40), an extracellular metalloprotease from *Serratia*.
- Alkaline metalloproteinase from *Pseudomonas aeruginosa* (gene aprA).
- 15 - Secreted proteases A, B, C and G from *Erwinia chrysanthemi*.
- Yeast hypothetical protein YIL108w.

Family M10B

- 20 - Mammalian extracellular matrix metalloproteinases (known as matrixins) [5]: MMP-1 (EC 3.4.24.7) (interstitial collagenase), MMP-2 (EC 3.4.24.24) (72 Kd gelatinase), MMP-9 (EC 3.4.24.35) (92 Kd gelatinase), MMP-7 (EC 3.4.24.23) (matrylisin), MMP-8 (EC 3.4.24.34) (neutrophil collagenase), MMP-3 (EC 3.4.24.17) (stromelysin-1), MMP-10 (EC 3.4.24.22) (stromelysin-2), and MMP-11 (stromelysin-3), MMP-12 (EC 3.4.24.65) (macrophage metalloelastase).
- 25 - Sea urchin hatching enzyme (envelysin) (EC 3.4.24.12). A protease that allows the embryo to digest the protective envelope derived from the egg extracellular matrix.
- Soybean metalloendoproteinase 1.

Family M11

- 30 - *Chlamydomonas reinhardtii* gamete lytic enzyme (GLE).

Family M12A

- Astacin (EC 3.4.24.21), a crayfish endoprotease.
- Meprin A (EC 3.4.24.18), a mammalian kidney and intestinal brush border

metalloendopeptidase.

- Bone morphogenic protein 1 (BMP-1), a protein which induces cartilage and bone formation and which expresses metalloendopeptidase activity. The *Drosophila* homolog of BMP-1 is the dorsal-ventral patterning protein tolloid.

5 - Blastula protease 10 (BP10) from *Paracentrotus lividus* and the related protein SpAN from *Strongylocentrotus purpuratus*.

- *Caenorhabditis elegans* protein toh-2.

- *Caenorhabditis elegans* hypothetical protein F42A10.8.

10 - Choriolytins L and H (EC 3.4.24.67) (also known as embryonic hatching proteins LCE and HCE) from the fish *Oryzias latipes*. These proteases participate in the breakdown of the egg envelope, which is derived from the egg extracellular matrix, at the time of hatching.

Family M12B

15 - Snake venom metalloproteinases [6]. This subfamily mostly groups proteases that act in hemorrhage. Examples are: adamalysin II (EC 3.4.24.46), atrolysin C/D (EC 3.4.24.42), atrolysin E (EC 3.4.24.44), fibrolase (EC 3.4.24.72), trimereylisin I (EC 3.4.25.52) and II (EC 3.4.25.53).

- Mouse cell surface antigen MS2.

20 Family M13

- Mammalian neprilysin (EC 3.4.24.11) (neutral endopeptidase) (NEP).

- Endothelin-converting enzyme 1 (EC 3.4.24.71) (ECE-1), which processes the precursor of endothelin to release the active peptide.

25 - Kell blood group glycoprotein, a major antigenic protein of erythrocytes. The Kell protein is very probably a zinc endopeptidase.

- Peptidase O from *Lactococcus lactis* (gene pepO).

Family M27

30 - Clostridial neurotoxins, including tetanus toxin (TeTx) and the various botulinum toxins (BoNT). These toxins are zinc proteases that block neurotransmitter release by proteolytic cleavage of synaptic proteins such as synaptobrevins, syntaxin and SNAP-25 [7,8].

Family M30

- *Staphylococcus hyicus* neutral metalloprotease.

Family M32

- 5 - Thermostable carboxypeptidase 1 (EC 3.4.17.19) (carboxypeptidase Taq), an enzyme from *Thermus aquaticus* which is most active at high temperature.

Family M34

- 10 - Lethal factor (LF) from *Bacillus anthracis*, one of the three proteins composing the anthrax toxin.

Family M35

- 15 - Deuterolysin (EC 3.4.24.39) from *Penicillium citrinum* and related proteases from various species of *Aspergillus*.

Family M36

- Extracellular elastinolytic metalloproteinases from *Aspergillus*.

- 20 From the tertiary structure of thermolysin, the position of the residues acting as zinc ligands and those involved in the catalytic activity are known. Two of the zinc ligands are histidines which are very close together in the sequence; C-terminal to the first histidine is a glutamic acid residue which acts as a nucleophile and promotes the attack of a water molecule on the carbonyl carbon of the substrate. A signature pattern which includes the two histidine and the glutamic acid residues is sufficient to detect this superfamily of
25 proteins.

Consensus pattern[GSTALIVN]-x(2)-H-E-[LIVMFYW]-{DEHRKP}-H-x-
[LIVMFYWGSPQ]

[The two H's are zinc ligands] [E is the active site residue]

- 30 Sequences known to belong to this class detected by the patternALL, except for members of families M5, M7 and M11.

Other sequence(s) detected in SWISS-PROT57; including *Neurospora crassa* conidiation-specific protein 13 which could be a zinc-protease.

[1]Jongeneel C.V., Bouvier J., Bairoch A. FEBS Lett. 242:211-214(1989).

- [2]Murphy G.J.P., Murphy G., Reynolds J.J. FEBS Lett. 289:4-7(1991).
 [3]Bode W., Grams F., Reinemer P., Gomis-Rueth F.-X., Baumann U., McKay D.B., Stoecker W. Zoology 99:237-246(1996).
 [4]Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).
 5 [5]Woessner J. Jr. FASEB J. 5:2145-2154(1991).
 [6]Hite L.A., Fox J.W., Bjarnason J.B. Biol. Chem. Hoppe-Seyler 373:381-385(1992).
 [7]Montecucco C., Schiavo G. Trends Biochem. Sci. 18:324-327(1993).
 [8]Niemann H., Blasi J., Jahn R. Trends Cell Biol. 4:179-185(1994).

10 985. PHO4: Phosphate transporter family

This family includes PHO-4 from *Neurospora crassa* which is a Na(+)-phosphate symporter [1]. This family also contains the leukemia virus receptor Swiss:Q08344. Number of members: 41

- 15 [1] Medline: 95249577 Repressible cation-phosphate symporters in *Neurospora crassa*.
 Versaw WK, Metzberg RL; Proc Natl Acad Sci U S A 1995;92:3884-3887.

986. Photosynthetic reaction center proteins signature (photoRC)
 PROSITE cross-reference(s): PS00244; REACTION_CENTER

20

In the photosynthetic reaction center of purple bacteria, two homologous integral membrane proteins, L(ight) and M(edium), are known to be essential to the light-mediated water-splitting process. In the photosystem II of eukaryotic chloroplasts two related proteins are involved: the D1 (psbA) and D2 proteins (psbD). These four types of protein
 25 probably evolved from a common ancestor [see 1,2 for recent reviews].

- A signature pattern was developed which include two conserved histidine residues. In L and M chains, the first histidine is a ligand of the magnesium ion of the special pair bacteriochlorophyll, the second is a ligand of a ferrous non-heme iron atom. In photosystem
 30 II these two histidines are thought to play a similar role.

Consensus pattern[NOH]-x(4)-P-x-H-x(2)-[SAG]-x(11)-[SAGC]-x-H-[SAG](2)
 [The first H is a magnesium ligand] [The second H is a iron ligand]
 Sequences known to belong to this class detected by the patternALL, except

for broad bean psbA which has Gln instead of the second His.

[1]Michel H., Deisenhofer J. *Biochemistry* 27:1-7(1988).

[2]Barber J. *Trends Biochem. Sci.* 12:321-326(1987).

5

987. phytochrome: Phytochrome region

This family contains a region specific to phytochrome proteins. Number of members:

145

10 988. PI3K_C2: C2 domain

Phosphoinositide 3-kinase region postulated to contain a C2 domain. Outlier of C2 family.

Number of members: 39

15

[1] Medline: 97388296 Using structure to define the function of phosphoinositide 3-kinase family members. Domin J, Waterfield MD; *FEBS Lett* 1997;410:91-95.

[2] Medline: 97398940 Phosphoinositide 3-kinases: a conserved family of signal transducers. Vanhaesebroeck B, Leever SJ, Panayotou G, Waterfield MD; *Trends Biochem Sci* 1997;22:267-272.

20

989. PI3Ka: Phosphoinositide 3-kinase family, accessory domain (PIK domain)

PIK domain is conserved in all PI3 and PI4-kinases. Its role is unclear but it has been suggested [2] to be involved in substrate presentation.

Number of members: 47

25

[1] Medline: 97388296 Using structure to define the function of phosphoinositide 3-kinase family members. Domin J, Waterfield MD; *FEBS Lett* 1997;410:91-95.

[2] Medline: 94069320 Phosphatidylinositol 4-kinase: gene structure and requirement for yeast cell viability. Flanagan CA, Schnieders EA, Emerick AW, Kunisawa R, Admon A, Thorner J; *Science* 1993;262:1444-1448.

30

990. P-II protein signatures

PROSITE cross-reference(s): PS00496; PII_GLNB_UMP, PS00638; PII_GLNB_CTER

The P-II protein (gene *glnB*) is a bacterial protein important for the control of glutamine synthetase [1,2,3]. In nitrogen-limiting conditions, when the ratio of glutamine to 2-ketoglutarate decreases, P-II is uridylylated on a tyrosine residue to form P-II-UMP. P-II-UMP allows the deadenylation of glutamine synthetase (GS), thus activating the enzyme.

- 5 Conversely, in nitrogen excess, P-II-UMP is deuridylylated and then promotes the adenylation of GS. P-II also indirectly controls the transcription of the GS gene (*glnA*) by preventing NR-II (*ntrB*) to phosphorylate NR-I (*ntrC*) which is the transcriptional activator of *glnA*. Once P-II is uridylylated, these events are reversed.

- 10 P-II is a protein of about 110 amino acid residues extremely well conserved. The tyrosine which is uridylylated is located in the central part of the protein.

In cyanobacteria, P-II seems to be phosphorylated on a serine residue rather than being uridylylated.

- 15 In methanogenic archaeobacteria, the nitrogenase iron protein gene (*nifH*) is followed by two open reading frames highly similar to the eubacterial P-II protein [4]. These proteins could be involved in the regulation of nitrogen fixation.

- 20 In the red alga, *Porphyra purpurea*, there is a *glnB* homolog encoded in the chloroplast genome.

Other proteins highly similar to *glnB* are:

- 25 - *Bacillus subtilis* protein *nrgB* [5].
- *Escherichia coli* hypothetical protein *ybaI* [6].

- Two signature patterns were developed for P-II protein. The first one is a conserved stretch (in eubacteria) of six residues which contains the uridylylated tyrosine, the other
30 is derived from a conserved region in the C-terminal part of the P-II protein.

Consensus pattern Y-[KR]-G-[AS]-[AE]-Y [The second Y is uridylylated]
Sequences known to belong to this class detected by the pattern ALL *glnB*'s
from eubacteria.

Consensus pattern[ST]-x(3)-G-[DY]-G-[KR]-[IV]-[FW]-[LIVM]-x(2)-[LIVM]

[1]Magasanik B. Biochimie 71:1005-1012(1989).

[2]Holtel A., Merrick M. Mol. Gen. Genet. 215:134-138(1988).

5 [3]Cheah E., Carr P.D., Suffolk P.M., Vasuvedan S.G., Dixon N.E., Ollis D.L. Structure 2:981-990(1994).

[4]Sibold L., Henriquet M., Possot O., Aubert J.-P. Res. Microbiol. 142:5-12(1991).

[5]Wray L.V. Jr., Atkinson M.R., Fisher S.H. J. Bacteriol. 176:108-114(1994).

[6]Allikmets R., Gerrard B.C., Court D., Dean M.C. Gene 136:231-236(1993).

10

991. PIP5K: Phosphatidylinositol-4-phosphate 5-Kinase

This family contains a region from the common kinase core found in the type I phosphatidylinositol-4-phosphate 5-kinase (PIP5K) family as described in [1]. The family consists of various type I, II and III PIP5K enzymes. PIP5K catalyses the formation of phosphoinositol-4,5-bisphosphate via the phosphorylation of phosphatidylinositol-4-phosphate a precursor in the phosphoinositide signaling pathway. Number of members: 33

15

[1] Medline: 98204859. Type I phosphatidylinositol-4-phosphate 5-kinases. Cloning of the third isoform and deletion/substitution analysis of members of this novel lipid kinase family.

20

Ishihara H, Shibasaki Y, Kizuki N, Wada T, Yazaki Y, Asano T, Oka Y; J Biol Chem 1998;273:8741-8748.

[2] Medline: 97115834 Type I phosphatidylinositol-4-phosphate 5-kinases are distinct members of this novel lipid kinase family. Loijens JC, Anderson RA; J Biol Chem 1996 20;271:32937-32943.

25

992. PolyA_pol: Poly A polymerase family

This family includes nucleic acid independent RNA polymerases, such as Poly(A) polymerase, which adds the poly (A) tail to mRNA EC:2.7.7.19. This family also includes the tRNA nucleotidyltransferase that adds the CCA to the 3' of the tRNA

30

EC:2.7.7.25. Number of members: 31

[1] Medline: 93066242 Identification of the gene for an Escherichia coli poly(A) polymerase. Cao GJ, Sarkar N; Proc Natl Acad Sci U S A 1992;89:10380-10384.

993. Photosystem I psaA and psaB proteins signature (psaA_psaB)

PROSITE cross-reference(s)PS00419; PHOTOSYSTEM_I_PSAAB

Photosystem I (PSI) [1] is an integral membrane protein complex that uses light energy to mediate electron transfer from plastocyanin to ferredoxin. PSI is found in the chloroplast of plants and cyanobacteria. The electron transfer components of the reaction center of PSI are a primary electron donor P-700 (chlorophyll dimer) and five electron acceptors: A0 (chlorophyll), A1 (a phylloquinone) and three 4Fe-4S iron-sulfur centers: Fx, Fa, and Fb.

PsaA and psaB, two closely related proteins, are involved in the binding of P700, A0, A1, and Fx. psaA and psaB are both integral membrane proteins of 730 to 750 amino acids that seem to contain 11 transmembrane segments. The Fx 4Fe-4S iron-sulfur center is bound by four cysteines; two of these cysteines are provided by the psaA protein and the two others by psaB. The two cysteines in both proteins are proximal and located in a loop between the ninth and tenth transmembrane segments. A leucine zipper motif seems to be present [2] downstream of the cysteines and could contribute to dimerization of psaA/psaB.

The signature pattern for these proteins is based on the perfectly conserved region that includes the two iron-sulfur binding cysteines.

Consensus pattern C-D-G-P-G-R-G-G-T-C [The two C's bind the iron-sulfur center]

[1] Golbeck J.H. Biochim. Biophys. Acta 895:167-204(1987).

[2] Webber A.N., Malkin R. FEBS Lett. 264:1-14(1990).

994. PSBH: Photosystem II 10 kDa phosphoprotein

This protein is phosphorylated in a light dependent reaction.

Number of members: 20

995. PsbJ

This family consists of the photosystem II reaction center protein PsbJ from plants and Cyanobacteria. In Synechocystis sp. PCC 6803 PsbJ regulates the number of photosystem II centers in thylakoid membranes, it is a predicted 4kDa protein with one membrane spanning domain [1]. Number of members: 20

[1] Medline: 93131892. Genetic and immunological analyses of the cyanobacterium *Synechocystis* sp. PCC 6803 show that the protein encoded by the *psbJ* gene regulates the number of photosystem II centers in thylakoid membranes. Lind LK, Shukla VK, Nyhus KJ, Pakrasi HB; J Biol Chem 1993;268:1575-1579.

996. PSBT: Photosystem II reaction centre T protein

The exact function of this protein is unknown. It probably consists of a single transmembrane spanning helix. The Swiss:P37256 protein, appears to be (i) a novel photosystem II subunit and (ii) required for maintaining optimal photosystem II activity under adverse growth conditions [1]. Number of members: 17

[1] Medline: 94298765. The chloroplast *ycf8* open reading frame encodes a photosystem II polypeptide which maintains photosynthetic activity under adverse growth conditions. Monod C, Takahashi Y, Goldschmidt-Clermont M, Rochaix JD; EMBO J 1994;13:2747-2754.

997. PSI_8. PHOTOSYSTEM I REACTION CENTRE SUBUNIT VIII. Synonym(s)PSI-I. Gene name(s)PSAI. From *Hordeum vulgare* (Barley). Encoded on Chloroplast. Taxonomy Eukaryota; Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; *Hordeum*. MAY HELP IN THE ORGANIZATION OF THE PSAL SUBUNIT. BELONGS TO THE PSAL FAMILY.

[1] SEQUENCE FROM N.A. MEDLINE; 90036933. Scheller H.V., Okkels J.S., Hoej P.B., Svendsen I., Roepstorff P., Moeller B.L.; "The primary structure of a 4.0-kDa photosystem I polypeptide encoded by the chloroplast *psaI* gene."; J. Biol. Chem. 264:18402-18406(1989).

998. PSI_PsaJ: Photosystem I reaction centre subunit IX / PsaJ

This family consists of the photosystem I reaction centre subunit IX or PsaJ from various organisms including *Synechocystis* sp. (strain pcc 6803), *Pinus thunbergii* (green pine) and *Zea mays* (maize). PsaJ Swiss:P19443 is a small 4.4kDa, chloroplastal encoded, hydrophobic subunit of the photosystem I reaction complex its function is not yet fully understood [1]. PsaJ can be cross-linked to PsaF Swiss:P12356 and has a single predicted transmembrane

domain it has a proposed role in maintaining Psaf in the correct orientation to allow for fast electron transfer from soluble donor proteins to P700+ [1]. Number of members: 18

[1] Medline: 99238330. A large fraction of Psaf is nonfunctional in photosystem I complexes lacking the Psaj subunit. Fischer N, Boudreau E, Hippler M, Drepper F, Haehnel W, Roach J; Biochemistry 1999;38:5546-5552.

[2] Medline: 93252282. Genes encoding eleven subunits of photosystem I from the thermophilic cyanobacterium Synechococcus sp. Muhlenhoff U, Haehnel W, Witt H, Hermann RG; Gene 1993;127:71-78.

999. PSII. Protein namePHOTOSYSTEM II P680 CHLOROPHYLL A APOPROTEIN. Synonym(s)CP-47 PROTEIN. Gene name(s)PSBB. From Hordeum vulgare (Barley), Encoded on Chloroplast. Taxonomy Eukaryota; Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Hordeum.

FUNCTION: THIS PROTEIN CONJUGATES WITH CHLOROPHYLL & CATALYZES THE PRIMARY LIGHT-INDUCED PHOTOCHEMICAL PROCESSES OF PHOTOSYSTEM II. SUBCELLULAR LOCATION: CHLOROPLAST THYLAKOID MEMBRANE. SIMILARITY: BELONGS TO THE PSBB / PSBC FAMILY.

[1] SEQUENCE FROM N.A. STRAIN=CV. SABARLIS; MEDLINE: 89240047. Andreeva A.V., Buryakova A.A., Reverdatto S.V., Chakhmakhcheva O.G., Efimov V.A.; "Nucleotide sequence of the 5.2 kbp barley chloroplast DNA fragment, containing psbB-psbH-petB-petD gene cluster."; Nucleic Acids Res. 17:2859-2860(1989).

[2] SEQUENCE FROM N.A. STRAIN=CV. SABARLIS; MEDLINE: 92207253. Efimov V.A., Andreeva A.V., Reverdatto S.V., Chakhmakhcheva O.G.; "Photosystem II of rye. Nucleotide sequence of the psbB, psbC, psbE, psbF, psbH genes of rye and chloroplast DNA regions adjacent to them."; Bioorg. Khim. 17:1369-1385(1991).

[3] SEQUENCE OF 411-420. Hinz U.G.; "Isolation of the photosystem II reaction center complex from barley. Characterization by circular dichroism spectroscopy and amino acid sequencing."; Carlsberg Res. Commun. 50:285-298(1985).

1000. QRPTase. Quinolate phosphoribosyl transferase.

Quinolate phosphoribosyl transferase (QRPTase) or nicotinate-nucleotide pyrophosphorylase EC:2.4.2.19 is involved in the de novo synthesis of NAD in both

prokaryotes and eukaryotes. It catalyses the reaction of quinolinic acid with 5-phosphoribosyl-1-pyrophosphate (PRPP) in the presence of Mg^{2+} to give rise to nicotinic acid mononucleotide (NaMN), pyrophosphate and carbon dioxide [1,2]. Number of members: 26.

[1]Medline: 97169443. A new function for a common fold: the crystal structure of quinolinic acid phosphoribosyltransferase. Eads JC, Ozturk D, Wexler TB, Grubmeyer C, Sacchettini JC; Structure 1997;5:47-58.

[2]Medline: 96139309. The sequencing expression, purification, and steady-state kinetic analysis of quinolinate phosphoribosyl transferase from *Escherichia coli*. Bhatia R, Calvo KC; Arch Biochem Biophys 1996;325:270-278.

1001. R3H domain

The name of the R3H domain comes from the characteristic spacing of the most conserved arginine and histidine residues. The function of the domain is predicted to be binding ssDNA. Number of members: 28

[1]Medline: 99003905 The R3H motif: a domain that binds single-stranded nucleic acids. Grishin NV; Trends Biochem Sci 1998;23:329-330.

1002. recF protein signatures (RecF)

The prokaryotic protein recF [1,2] is a single-stranded DNA-binding protein which also probably binds ATP. RecF is involved in DNA metabolism; it is required for recombinational DNA repair and for induction of the SOS response. RecF is a protein of about 350 to 370 amino acid residues; there is a conserved ATP-binding site motif 'A' (P-loop) in the N-terminal section of the protein as well as two other conserved regions, one located in the central section, and the other in the C-terminal section. Signature patterns were derived from these two regions.

Consensus pattern [LIVM]-x(4)-[LIF]-x(6)-[LIF]-[LVF]-x-[GE]-[GSTAD]-[PA]- x(2)-R-R-x-[FYW]-[LIVMF]-D Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern[LIVMFY](2)-x-D-x(2,3)-[SA]-[EH]-L-D-x(2)-[KRH]-x(3)-L Sequences known to belong to this class detected by the patternALL, except for T. palidum recF.

[1] Sandler S.J., Chackerian B., Li J.T., Clark A.J. Nucleic Acids Res. 20:839-845(1992).

5 [2] Alonso J.C., Fisher L.M.; Mol. Gen. Genet. 246:680-686(1995).

1003. RibD C-terminal domain (RibD_C)

The function of this domain is not known, but it is thought to be involved in riboflavin biosynthesis. This domain is found in the C terminus of RibD/RibG Swiss:P25539, in combination with dCMP_cyt_deam, as well as in isolation in some archaeobacterial proteins Swiss:P95872.

Number of members: 21

15 1004. Ribosomal protein L16 signatures (Ribosomal_L16)

Ribosomal protein L16 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L16 is known to bind directly the 23S rRNA and to be located at the A site of the peptidyltransferase center. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups:

- Eubacterial L16.
- Algal and plant chloroplast L16.
- Cyanelle L16.
- Plant mitochondrial L16.

25 L16 is a protein of 133 to 185 amino-acid residues. As signature patterns, we selected two conserved regions in the central section of these proteins.

Consensus pattern [KR](2)-x-[GSAC]-[KQVA]-[LIVM]-W-[LIVM]-[KR]-[LIVM]-[LFY]-[AP] Sequences known to belong to this class detected by the pattern ALL.

30 Consensus pattern R-M-G-x-[GR]-K-G-x(4)-[FWKR] Sequences known to belong to this class detected by the patternALL.

[1] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

1005. Ribosomal protein L32e signature (Ribosomal_L32E)

A number of eukaryotic and archaeobacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L32 [1].
- Drosophila RP49 [2].
- Trichoderma harzianum L32 [3].
- Yeast L32e (YBL092w).
- Archaeobacterial L32e [4].

These proteins have 135 to 240 amino-acid residues. As a signature pattern, a stretch of about 20 residues located in the N-terminal part of these proteins was selected.

Consensus pattern F-x-R-x(4)-[KR]-x(2)-[KR]-[LIVMF]-x(3,5)-W-R-[KR]-x(2)-G Sequences known to belong to this class detected by the pattern ALL.

- [1] Jacks C.M., Powaser C.B., Hackett P.B. Gene 74:565-570(1988).
- [2] Aguade M. Mol. Biol. Evol. 5:433-441(1988).
- [3] Lora J.M., Garcia I., Benitez T., Llobell A., Pintor-Toro J.A. Nucleic Acids Res. 21:3319-3319(1993).
- [4] Arndt E., Scholzen T., Kroemer W., Hatakeyama T., Kimura M. Biochimie 73:657-668(1991).

1006. (Ribosomal_S3) Ribosomal protein S3 signature

PROSITE: PDOC00474. PROSITE cross-reference(s) PS00548; RIBOSOMAL_S3

Ribosomal protein S3 is one of the proteins from the small ribosomal subunit.

In Escherichia coli, S3 is known to be involved in the binding of initiator Met-tRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups:

- Eubacterial S3.
- Algal and plant chloroplast S3.
- Cyanelle S3.
- Archaeobacterial S3.
- Plant mitochondrial S3.

-Vertebrate S3.

-Insect S3.

-Caenorhabditis elegans S3 (C23G10.3).

-Yeast S3 (Rp13).

- 5 S3 is a protein of 209 to 559 amino-acid residues. A conserved region located in the C-terminal section was selected as a signature pattern.

Consensus pattern[GSTA]-[KR]-x(6)-G-x-[LIVMT]-x(2)-[NQSCH]-x(1,3)-[LIVFCA]-x(3)-[LIV]-[DENQ]-x(7)-[LMT]-x(2)-G-x(2)-[GS]. Sequences known to belong to this class

- 10 detected by the patternALL, except for some mitochondrial S3.

[1]Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

1007. RimM - RimM

- 15 The RimM protein is essential for efficient processing of 16S rRNA [1]. The RimM protein was shown to have affinity for free ribosomal 30S subunits but not for 30S subunits in the 70S ribosomes [1]. Number of members: 14.

[1]Medline: 98083058. RimM and RbfA are essential for efficient processing of 16S rRNA in Escherichia coli. Bylund GO, Wipemo LC, Lundberg LA, Wikstrom PM; J Bacteriol 1998;180:73-82.

1008. RNA_pol_A - RNA polymerase alpha subunit

- !- RNA polymerases catalyse the DNA dependent polymerisation of RNA. Prokaryotes contain a single RNA polymerase compared to three in eukaryotes (not including mitochondrial and chloroplast polymerases).
- !- Members of this family include: A subunit from eukaryotes, gamma subunit from cyanobacteria, beta' subunit from eubacteria, A' subunit from archaeobacteria, B'' from chloroplasts. Number of members: 139.

[1]Medline: 97066998. Structural modules of the large subunits of RNA polymerase. Introducing archaeobacterial and chloroplast split sites in the beta and beta' subunits of Escherichia coli RNA polymerase. Severinov K, Mustaev A, Kukarin A, Muzzin O, Bass I, Darst SA, Goldfarb A; J Biol Chem 1996;271:27969-27974.

1009. RuBisCO_large - Ribulose biphosphate carboxylase large chain active site

PROSITE: PDOC00142; PROSITE cross-reference(s) PS00157; RUBISCO_LARGE

Ribulose biphosphate carboxylase (EC 4.1.1.39) (RuBisCO) [1,2] catalyzes the

5 initial step in Calvin's reductive pentose phosphate cycle in plants as well as purple and green bacteria. It consists of a large catalytic unit and a small subunit of undetermined function. In plants, the large subunit is coded by the chloroplastic genome while the small subunit is encoded in the nuclear genome. Molecular activation of RuBisCO by CO₂ involves the formation of a carbamate with the epsilon-amino group of a conserved lysine residue. This

10 carbamate is stabilized by a magnesium ion. One of the ligands of the magnesium ion is an aspartic acid residue close to the active site lysine [3]. A pattern was developed which includes both the active site residue and the metal ligand, and which is specific to RuBisCO large chains.

15 Consensus pattern G-x-[DN]-F-x-K-x-D-E [K is the active site residue] [The second D is a magnesium ligand]. Sequences known to belong to this class detected by the pattern ALL, except for Cheilopleuria biscuspis RuBisCO.

[1] Mizziorko H.M., Lorimer G.H. Annu. Rev. Biochem. 52:507-535(1983).

20 [2] Akazawa T., Takabe T., Kobayashi H. Trends Biochem. Sci. 9:380-383(1984).

[3] Andersson I., Knight S., Schneider G., Lindqvist Y., Lundqvist T., Branden C.-I., Lorimer G.H. Nature 337:229-234(1989).

1010. Rve - Integrase core domain

25 Integrase mediates integration of a DNA copy of the viral genome into the host chromosome. Integrase is composed of three domains. The amino-terminal domain is a zinc binding domain Integrase_Zn. This domain is the central catalytic domain. The carboxyl terminal domain that is a non-specific DNA binding domain integrase. The catalytic domain acts as an endonuclease when two nucleotides are removed from the 3' ends of the blunt-ended viral

30 DNA made by reverse transcription. This domain also catalyses the DNA strand transfer reaction of the 3' ends of the viral DNA to the 5' ends of the integration site [1]. Number of members: 694.

[1]Medline: 95099322. Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. Dyda F, Hickman AB, Jenkins TM, Engelman A, Craigie R, Davies DR; Science 1994;266:1981-1986.

5 1011. (SBP_bac_3) Bacterial extracellular solute-binding proteins, family 3 signature
PROSITE: PDOC00798. PROSITE cross-reference(s) PS01039; SBP_BACTERIAL_3

Bacterial high affinity transport systems are involved in active transport of solutes across the cytoplasmic membrane. The protein components of these traffic systems include one or two transmembrane protein components, one or two membrane-associated ATP-binding proteins (ABC transporters; see <PDOC00185>) and a high affinity periplasmic solute-binding protein. The later are thought to bind the substrate in the vicinity of the inner membrane, and to transfer it to a complex of inner membrane proteins for concentration into the cytoplasm.

15 In gram-positive bacteria which are surrounded by a single membrane and have therefore no periplasmic region the equivalent proteins are bound to the membrane via an N-terminal lipid anchor. These homolog proteins do not play an integral role in the transport process per se, but probably serve as receptors to trigger or initiate translocation of the solute through the membrane by binding to external sites of the integral membrane proteins of the efflux system.

20 In addition at least some solute-binding proteins function in the initiation of sensory transduction pathways.

On the basis of sequence similarities, the vast majority of these solute-binding proteins can be grouped [1] into eight families of clusters, which generally correlate with the nature of the solute bound.

25 Family 3 groups together specific amino acids and opine-binding periplasmic proteins and a periplasmic homolog with catalytic activity:

-Histidine-binding protein (gene hisJ) of Escherichia coli and related bacteria. An homologous lipoprotein exists in Neisseria gonorrhoeae.

30 -Lysine/arginine/ornithine-binding proteins (LAO) (gene argT) of Escherichia coli and related bacteria are involved in the same transport system than hisJ. Both solute-binding proteins interact with a common membrane-bound receptor hisP of the binding protein dependent transport system HisQMP.

-Glutamine-binding proteins (gene glnH) of Escherichia coli and Bacillus stearothermophilus.

-Glutamate-binding protein (gene *gluB*) of *Corynebacterium glutamicum*.

-Arginine-binding proteins *artI* and *artJ* of *Escherichia coli*.

-Nopaline-binding protein (gene *nocT*) from *Agrobacterium tumefaciens*.

-Octopine-binding protein (gene *occT*) from *Agrobacterium tumefaciens*.

5 -Major cell-binding factor (CBF1) (gene: *peb1A*) from *Campylobacter jejuni*.

-*Bacteroides nodosus* protein *aabA*.

-Cyclohexadienyl/rotenone dehydratase of *Pseudomonas aeruginosa*, a periplasmic enzyme which forms an alternative pathway for phenylalanine biosynthesis.

-*Escherichia coli* protein *fliY*.

10 -*Vibrio harveyi* protein *path*.

-*Escherichia coli* hypothetical protein *ydhW*.

-*Bacillus subtilis* hypothetical protein *yckB*.

-*Bacillus subtilis* hypothetical protein *yckK*.

15 The signature pattern is located near the N-terminus of the mature proteins.

Consensus pattern G-[FYIL]-[DE]-[LIVMT]-[DE]-[LIVMF]-x(3)-[LIVMA]-[VAGC]-x(2)-[LIVMAGN]

Sequences known to belong to this class detected by the pattern ALL.

20 [1] Tam R., Saier M.H. Jr. *Microbiol. Rev.* 57:320-346(1993).

1012. Sec7 - Sec7 domain

The Sec7 domain is a guanine-nucleotide-exchange-factor (GEF) for the arf family [2].

Number of members: 32.

25

[1] Medline: 98169075. Structure of the Sec7 domain of the Arf exchange factor. ARNO. Cherfils J, Menetrey J, Mathieu M, Le Bras G, Robineau S, Beraud-Dufour S, Antonny B, Chardin P; *Nature* 1998;392:101-105.

30

[2] Medline: 97100951. A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains. Chardin P, Paris S, Antonny B, Robineau S, Beraud-Dufour S, Jackson CL, Chabre M. *Nature* 1996;384:481-484.

1013. SecA_protein. SecA protein, amino terminal region

SecA protein binds to the plasma membrane where it interacts with proOmpA to support translocation of proOmpA through the membrane. SecA protein achieves this translocation, in association with SecY protein, in an ATP dependent manner. SecA possesses the ATPase activity. The carboxyl terminus has similarity with the helicase carboxyl terminus. See

- 5 Ribosomal_L5. Number of members: 45.

[1]Medline: 98309858. Amino-terminal region of SecA is involved in the function of SecG for protein translocation into Escherichia coli membrane vesicles. Mori H, Sugiyama H, Yamanaka M, Sato K, Tagaya M, Mizushima S; J Biochem (Tokyo) 1998;124:122-129.

- 10 [2]Medline: 89251629. SecA protein hydrolyzes ATP and is an essential component of the protein translocation ATPase of Escherichia coli. Lill R, Cunningham K, Brundage LA, Ito K, Oliver D, Wickner W; EMBO J 1989;8:961-966.

1014. Seedstore_2S - 2S seed storage family

- 15 Members of this family are composed of two chains (both included in the alignment), these are co-translated and later cleaved. The two chains are disulphide linked together. Number of members: 27.

[1]Medline: 97121264. 1H NMR assignment and global fold of napin BnIb, a representative
20 2S albumin seed protein. Rico M, Bruix M, Gonzalez C, Monsalve RI, Rodriguez R; Biochemistry 1996;35:15672-15682.

1015. Smr - Smr domain

- This family includes the Smr (Small MutS Related) proteins, and the C-terminal region of the
25 MutS2 protein. It has been suggested that this domain interacts with the MutS1 Swiss:P23909 protein in the case of Smr proteins and with the N-terminal MutS related region of MutS2 Swiss:P94545 [1]. Number of members: 14.

[1]Medline: 10431172. Smr: a bacterial and eukaryotic homologue of the C-terminal region
30 of the MutS2 family. Moreira D, Philippe H; Trends Biochem Sci 1999;24:298-300.

1016. (SSF) Sodium:solute symporter family signatures and profile

PROSITE: PDOC00429. PROSITE cross-reference(s)PS00456; NA_SOLUT_SYMP_1
PS00457; NA_SOLUT_SYMP_2 PS50283; NA_SOLUTE_SYMP_3

It has been shown [1,2] that integral membrane proteins that mediate the intake of a wide variety of molecules with the concomitant uptake of sodium ions (sodium symporters) can be grouped, on the basis of sequence and functional similarities into a number of distinct families. One of these families is known as the sodium:solute symporter family (SSF) and

currently consists of the following proteins:

- Mammalian Na⁺/glucose co-transporter.
- Mammalian Na⁺/myo-inositol co-transporter.
- Mammalian Na⁺/nucleoside co-transporter.
- Mammalian Na⁺/neutral amino acid co-transporter.
- Escherichia coli Na⁺/proline symporter (gene putP).
- Escherichia coli Na⁺/pantothenate symporter (gene panF).
- Escherichia coli hypothetical protein yidK.
- Escherichia coli hypothetical protein yjcG.
- Bacillus subtilis hypothetical protein ywCA (ipa-31R).

These integral membrane proteins are predicted to comprise at least ten membrane spanning domains. Two conserved regions were selected as signature patterns; the first one is located in the fourth transmembrane region and the second one in a loop between two transmembrane regions in the C-terminal part of these proteins.

Consensus pattern[GS]-x(2)-[LIY]-x(3)-[LIVMFYWSTAG](10)-[LIY]-[TAV]-x(2)-G-G-[LMF]-x-[SAP]. Sequences known to belong to this class detected by the patternALL. Consensus pattern[GAST]-[LIVM]-x(3)-[KR]-x(4)-G-A-x(2)-[GAS]-[LIVMGS]-[LIVMW]-[LIVMGAT]-G-x-[LIVMGA] Sequences known to belong to this class detected by the patternALL, except for E.coli yidK.

Note this documentation entry is linked to both a signature pattern and a profile. As the profile is much more sensitive than the pattern, you should use it if you have access to the necessary software tools to do so.

[1]Reizer J., Reizer A., Saier M.H. Jr. Res. Microbiol. 141:1069-1072(1991).

[2]Reizer J., Reizer A., Saier M.H. Jr. Biochim. Biophys. Acta 1197:133-136(1994).

1017. SurE - Survival protein SurE

E. coli cells with the surE gene disrupted are found to survive poorly in stationary phase [1].

It is suggested that SurE may be involved in stress response. Yeast also contains a member of

the family Swiss:P38254. Swiss:P30887 can complement a mutation in acid phosphatase, suggesting that members of this family could be phosphatases. Number of members: 17.

[1]Medline: 95014035. A new gene involved in stationary-phase survival located at 59 minutes on the Escherichia coli chromosome. Li C, Ichikawa JK, Ravetto JJ, Kuo HC, Fu JC, Clarke S; J Bacteriol 1994;176:6015-6022.

[2]Medline: 93046805. Complementation of Saccharomyces cerevisiae acid phosphatase mutation by a genomic sequence from the yeast Yarrowia lipolytica identifies a new phosphatase. Treton BY, Le Dall MT, Gaillardin CM; Curr Genet 1992;22:345-355.

1018. Synuclein - Synuclein

There are three types of synucleins in humans, these are called alpha, beta and gamma. Alpha synuclein has been found mutated in families with autosomal dominant Parkinson's disease. A peptide of alpha synuclein has also been found in amyloid plaques in Alzheimer's patients. Number of members: 12.

[1]Medline: 98424410. The synuclein family. Lavedan C; Genome Res 1998;8:871-880.

1019. (T-box) T-box domain signatures

PROSITE: PDOC00972. PROSITE cross-reference(s) PS01283; TBOX_1 PS01264; TBOX_2

A number of eukaryotic DNA-binding proteins contain a domain of about 170 to 190 amino acids known as the T-box domain [1,2,3] and which probably binds DNA. The T-box has first been found in the mice T locus (Brachyury) protein, a transcription factor involved in mesoderm differentiation. It has since been found in the following proteins:

- Vertebrate and invertebrate homologs of the T protein.
- Mammalian proteins TBX1 to TBX6.
- Mammalian protein TBRI which is expressed specifically in brain.
- Xenopus laevis eomesodermin (eomes).
- Xenopus laevis Vegt (or Antipodean), a transcription factor that activates the expression of wnt-8, eomes and Brachyury.
- Chicken TbxT.
- Drosophila protein optomotor-blind (omb).
- Drosophila protein brachyenteron (byn) (also known as Trg), which is

required for the specification of the hindgut and anal pads.

-Drosophila protein H15.

-Caenorhabditis elegans protein tbx-12.

-Caenorhabditis elegans hypothetical proteins F21H11.3, F40H6.4, T07C4.2, T07C4.6 and

5 ZK177.10.

Two conserved regions were selected as signature patterns for the T-domain. The first region corresponds to the N-terminal of the domain and the second one to the central part.

Consensus pattern L-W-x(2)-[FC]-x(3,4)-[NT]-E-M-[LIV](2)-T-x(2)-G-[RG]-[KRQ]

10 Sequences known to belong to this class detected by the pattern ALL, except for C.elegans ZK177.10.

Consensus pattern [LIVMYW]-H-[PADH]-[DEN]-[GS]-x(3)-G-x(2)-W-M-x(3)-[IVA]-x- F

Sequences known to belong to this class detected by the pattern ALL, except for C.elegans tbx-12, ZK177.10 and Drosophila H15.

15

[1] Bollag R.J., Siegfried Z., Cebra-Thomas J.A., Garvey N., Davison E.M., Silver L.M. Nat. Genet. 7:383-389(1994).

[2] Agulnik S.I., Garvey N., Hancock S., Ruvinsky I., Chapman D.L., Agulnik I., Bollag R.J., Papaioannou V.E., Silver L.M. Genetics 144:249-254(1996).

20

[3] Papaioannou V.E. Trends Genet. 13:212-213(1997).

1020. Toprim - Toprim domain

This is a conserved region from DNA primase. This corresponds to the Toprim domain common to DnaG primases, topoisomerases, OLD family nucleases and RecR proteins [1].

25

Both DnaG motifs IV and V are present in the alignment, the DxD (V) motif may be involved in Mg²⁺ binding and mutations to the conserved glutamate (IV) completely abolish DnaG type primase activity [1]. DNA primase EC:2.7.7.6 is a nucleotidyltransferase it synthesizes the oligoribonucleotide primers required for DNA replication on the lagging strand of the replication fork; it can also prime the leading strand and has been implicated in cell division

30

[2]. Number of members: 133.

[1] Medline: 98391745. Toprim--a conserved catalytic domain in type IA and II

topoisomerases, DnaG-type primases, OLD family nucleases and RecR proteins. Aravind L, Leipe DD, Koonin EV; Nucleic Acids Res 1998;26:4205-4213.

[2]Medline: 97368180. Cloning and analysis of the dnaG gene encoding *Pseudomonas putida* DNA primase. Szafranski P, Smith CL, Cantor CR; *Biochim Biophys Acta* 1997;1352:243-248.

[3]Medline: 94124015. The *Haemophilus influenzae* dnaG sequence and conserved bacterial primase motifs. Versalovic J, Lupski JR; *Gene* 1993;136:281-286.

1021. TraB - TraB family

pAD1 is a hemolysin/bacteriocin plasmid originally identified in *Enterococcus faecalis* DS16. It encodes a mating response to a peptide sex pheromone, cAD1, secreted by recipient bacteria. Once the plasmid pAD1 is acquired, production of the pheromone ceases--a trait related in part to a determinant designated traB. However a related protein is found in *C. elegans* Swiss:Q94217, suggesting that members of the TraB family have some more general function. Number of members: 12.

[1]Medline: 94302142. Characterization of the determinant (traB) encoding sex pheromone shutdown by the hemolysin/bacteriocin plasmid pAD1 in *Enterococcus faecalis*. An FY, Clewell DB; *Plasmid* 1994;31:215-221.

1022. (Transpo_mutator) Transposases, Mutator family, signature

PROSITE: PDOC00770. PROSITE cross-reference(s) PS01007;
TRANSPOSASE_MUTATOR

Autonomous mobile genetic elements such as transposon or insertion sequences (IS) encode an enzyme, called transposase, required for excising and inserting the mobile element. On the basis of sequence similarities, transposases can be grouped into various families. One of these families has been shown [1,2,3,E1] to consist of transposases from the following elements:

- Mutator from Maize.
- Is1201 from *Lactobacillus helveticus*.
- Is905 from *Lactococcus lactis*.
- Is1081 from *Mycobacterium bovis*.
- Is6120 from *Mycobacterium smegmatis*.
- Is406 from *Pseudomonas cepacia*.
- IsRm3 from *Rhizobium meliloti*.
- IsRm5 from *Rhizobium meliloti*.

-Is256 from *Staphylococcus aureus*.

-IsT2 from *Thiobacillus ferrooxidans*.

The maize Mutator transposase (MudrA) is a protein of 823 amino acids; the bacterial transposases listed above are proteins of 300 to 420 amino acids. These proteins contain a conserved domain of about 130 residues; a signature pattern was derived from the most conserved part of this domain.

Consensus pattern D-x(3)-G-[LIVMF]-x(6)-[STAV]-[LIVMFYW]-[PT]-x-[STAV]-x(2)-[QR]-x-C-x(2)-H. Sequences known to belong to this class detected by the pattern ALL.

[1] Eisen J.A., Benito M.-I., Walbot V. *Nucleic Acids Res.* 22:2634-2636(1994).

[2] Guilhot C., Gicquel B., Davies J., Martin C. *Mol. Microbiol.* 6:107-113(1992).

[3] Wood M.S., Byrne A., Lessie T.G. *Gene* 105:101-105(1991).

1023. Transposase_8 - Transposase

Transposase proteins are necessary for efficient DNA transposition. This family consists of various *E. coli* insertion elements and other bacterial transposases some of which are members of the IS3 family. Number of members: 58.

[1] Medline: 97324595. Genetic organization and transposition properties of IS511. D. A. Mullin, D. L. Zies, A. H. Mullin, N. Caballera & B. Ely; *Mol Gen Genet* 1997;254:456-463.

[2] Medline: 97128810. The use of an improved transposon mutagenesis system for DNA sequencing leads to the characterization of a new insertion sequence of *Streptomyces lividans* 66. J. Fischer, H. Maier, P. Viell & J. Altenbuchner; *Gene* 1996;180:81-89.

[3] Medline: 97074647. Identification and nucleotide sequence of *Rhizobium meliloti* insertion sequence ISRm6, a small transposable element that belongs to the IS3 family. S. Zekri & N. Toro; *Gene* 1996;175:43-48.

1024. tRNA_int_endo - tRNA intron endonuclease

Members of this family cleave pre tRNA at the 5' and 3' splice sites to release the intron EC:3.1.27.9, Number of members: 8.

[1]Medline: 97344075. Properties of *H. volcanii* tRNA intron endonuclease reveal a relationship between the archaeal and eucaryal tRNA intron processing systems. Kleman-Leyer K, Armbruster DW, Daniels CJ; Cell 1997;89:839-847.

5 1025. Urease - Urease signatures

PROSITE: PDOC00133PROSITE cross-reference(s) PS01120; UREASE_1 PS00145; UREASE_2

Urease (EC 3.5.1.5) is a nickel-binding enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia [1]. Historically, it was the first enzyme to be crystallized (in
10 1926). It is mainly found in plant seeds, microorganisms and invertebrates. In plants, urease is a hexamer of identical chains. In bacteria [2], it consists of either two or three different subunits (alpha, beta and gamma).

Urease binds two nickel ions per subunit; four histidine, an aspartate and a carbamated-lysine serve as ligands to these metals; an additional histidine is involved in the
15 catalytic mechanism [3].

As signatures for this enzyme, a region that contains two histidine that bind one of the nickel ions and the region of the active site histidine was selected.

Consensus pattern T-[AY]-[GA]-[GAT]-[LIVM]-D-x-H-[LIVM]-H-x(3)-P [The two H's bind
20 nickel]. Sequences known to belong to this class detected by the patternALL.

Consensus pattern[LIVM](2)-[CT]-H-[HN]-L-x(3)-[LIVM]-x(2)-D-[LIVM]-x-F-A [H is the active site residue]. Sequences known to belong to this class detected by the patternALL.

[1]Takishima K., Suga T., Mamiya G. Eur. J. Biochem. 175:151-165(1988).

25 [2]Mobley H.L.T., Husinger R.P. Microbiol. Rev. 53:85-108(1989).

[3]Jabri E., Carr M.B., Hausinger R.P., Karplus P.A. Science 268:998-1004(1995).

1026. Urease_beta - Urease beta subunit.

This subunit is known as alpha in *Helicobacter*. Number of members: 35.

[1]Medline: 95273988. The crystal structure of urease from *Klebsiella aerogenes*. Jabri E, Carr MB, Hausinger RP, Karplus PA; Science 1995;268:998-1004.

1027. UvrD-helicase - UvrD/REP helicase

The Rep family helicases are composed of four structural domains. The Rep family function as dimers. REP helicases catalyse ATP dependent unwinding of double stranded DNA to single stranded DNA. Swiss:P23478, Swiss:P08394 have large insertions near to the carboxy-terminus relative to other members of the family. Number of members: 52.

5

[1] Medline: 97433075. Major domain swiveling revealed by the crystal structures of complexes of E. coli Rep helicase bound to single-stranded DNA and ADP. Korolev S, Hsieh J, Gauss GH, Lohman TM, Waksman G; Cell 1997;90:635-647.

10 1028. V-type ATPase 116kDa subunit family (V_ATPase_sub_a)

This family consists of the 116kDa V-type ATPase (vacuolar (H⁺)-ATPases) subunits, as well as V-type ATP synthase subunit i. The V-type ATPases family are proton pumps that acidify intracellular compartments in eukaryotic cells for example yeast central vacuoles, clathrin-coated and synaptic vesicles. They have important roles in membrane trafficking processes [1]. The 116kDa subunit (subunit a) in the V-type ATPase is part of the V0 functional domain responsible for proton transport. The a subunit is a transmembrane glycoprotein with multiple putative transmembrane helices. It has a hydrophilic amino terminal and a hydrophobic carboxy terminal [1,2]. It has roles in proton transport and assembly of the V-type ATPase complex [1,2]. This subunit is encoded by two homologous gene in yeast VPH1 and STV1 [2].

15

20

Number of members: 27

[1] Forgac M; Medline: 99240666 Structure and properties of the vacuolar (H⁺)-ATPases." J Biol Chem 1999;274:12951-12954.

25

[2] Forgac M; Medline: 99270697 Structure and properties of the clathrin-coated vesicle and yeast vacuolar V-ATPases." J Bioenerg Biomembr 1999;31:57-65.

1029. Viral (Superfamily 1) RNA helicase (Viral_helicase1)

30 Number of members: 260

[1] Koonin EV, Dolja VV; Medline: 94094568 Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences." Crit Rev Biochem Mol Biol 1993;28:375-430.

1030. Vesicular monoamine transporter (VMAT)

This family consists of various vesicular amine transporters with 12 transmembrane helices.

- 5 These included vesicular acetylcholine transporters (VACHT) [3], and vesicular monoamine transporters (VMATs) [1,2] isoforms 1 adrenal and 2 brain (VMAT1 and VMAT2).

These proteins transport biogenic amines into synaptic vesicles or chromaffin granules [4].

- 10 VMATs pack monoamine neurotransmitters into secretory vesicles for regulated exocytotic release, they also protect against the parkinsonian neurotoxins MPP+ by transporting it into vesicles preventing it from acting on mitochondria [1].

Also in the family is *C. elegans* UNC-17 a putative vesicular acetylcholine transporter mutations in UNC-17 cause impaired neuromuscular function, giving rise to jerky or uncoordinated movement, [4].

Number of members: 15

[1] Krantz DE, Peter D, Liu Y, Edwards RH; Medline: 97197857 Phosphorylation of a vesicular monoamine transporter by casein kinase II." J Biol Chem 1997;272:6752-6759.

- 20 [2] Erickson JD, Varoqui H, Schafer MK, Modi W, Diebler MF, Weihe E, Rand J, Eiden LE, Bonner TI, Usdin TB; Medline: 94350930 Functional identification of a vesicular acetylcholine transporter and its expression from a 'cholinergic' gene locus." J Biol Chem 1994;269:21929-21932.

- 25 [3] Erickson JD, Schafer MK, Bonner TI, Eiden LE, Weihe E; Medline: 96209876 Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter." Proc Natl Acad Sci U S A 1996;93:5166-5171.

[4] Alfonso A, Grundahl K, Duerr JS, Han HP, Rand JB; Medline: 3342494 The *Caenorhabditis elegans* unc-17 gene: a putative vesicular acetylcholine transporter." Science 1993;261:617-619.

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1031. WW/rsp5/WWP domain signature and profile. Cross-reference(s): PS01159; WW_DOMAIN_1; PS50020; WW_DOMAIN_2

The WW domain [1-4,E1] (also known as *rsp5* or *WWP*) has been originally discovered as a short conserved region in a number of unrelated proteins, among them dystrophin, the gene responsible for Duchenne muscular dystrophy. The domain, which spans about 35 residues, is repeated up to 4 times in some proteins. It has been shown [5] to bind proteins with particular proline-motifs, [AP]-P-P-[AP]-Y, and thus resembles somewhat SH3 domains. It appears to contain beta-strands grouped around four conserved aromatic positions; generally Trp. The name WW or WWP derives from the presence of these Trp as well as that of a conserved Pro. It is frequently associated with other domains typical for proteins in signal transduction processes.

Proteins containing the WW domain are listed below.

--Dystrophin, a multidomain cytoskeletal protein. Its longest alternatively spliced form consists of an N-terminal actin-binding domain, followed by 24 spectrin-like repeats, a cysteine-rich calcium-binding domain and a C-terminal globular domain. Dystrophin forms tetramers and is thought to have multiple functions including involvement in membrane stability, transduction of contractile forces to the extracellular environment and organization of membrane specialization. Mutations in the dystrophin gene lead to muscular dystrophy of Duchenne or Becker type. Dystrophin contains one WW domain C-terminal of the spectrin-repeats.

--Utrophin, a dystrophin-like protein of unknown function.

--Vertebrate YAP protein is a substrate of an unknown serine kinase. It binds to the SH3 domain of the Yes oncoprotein via a proline-rich region. This protein appears in alternatively spliced isoforms, containing either one or two WW domains [6].

--Mouse NEDD-4 plays a role in the embryonic development and differentiation of the central nervous system. It contains 3 WW modules followed by a HECT domain. The human ortholog contains 4 WW domains, but the third WW domain is probably spliced resulting in an alternate NEDD-4 protein with only 3 WW modules [3].

--Yeast RSP5 is similar to NEDD-4 in its molecular organization. It contains an N-terminal C2 domain (see <PDOC00380>), followed by a histidine-rich region, 3 WW domains and a HECT domain.

--Rat FE65, a transcription-factor activator expressed preferentially in liver. The activator domain is located within the N-terminal 232 residues of FE65, which also contain the WW domain.

--Yeast ESS1/PTF1, a putative peptidyl prolyl cis-trans isomerase from family ppiC (see <PDOC00840>). A related protein, dodo (gene dod) exists in *Drosophila* and in mammals (gene PIN1).

--Tobacco DB10 protein. The WW domain is located N-terminal to the region with similarity to ATP-dependent RNA helicases.

--IQGAP, a human GTPase activating protein acting on ras. It contains an N-terminal domain similar to fly muscle mp20 protein and a C-terminal ras GTPase activator domain.

--Yeast pre-mRNA processing protein PRP40, *Caenorhabditis elegans* ZK1098.1 and fission yeast SpAC13C5.02 are related proteins with similarity to MYO2-type myosin, each

containing two WW-domains at the N-terminus.

--*Caenorhabditis elegans* hypothetical protein C38D4.5, which contains one WW module, a PH domain (see <PDOC50003>) and a C-terminal phosphatidylinositol 3-kinase domain.

--Yeast hypothetical protein YFL010c.

For the sensitive detection of WW domains, a profile was developed which spans the whole homology region as well as a pattern.

Description of pattern(s) and/or profile(s):

Consensus pattern W-x(9,11)-[VFY]-[FYW]-x(6,7)-[GSTNE]-[GSTQCR]-[FYW]-x(2)-P.

[1] Bork P., Sudol M. Trends Biochem. Sci. 19:531-533(1994).

[2] Andre B., Springael J.Y. Biochem. Biophys. Res. Commun. 205:1201-1205(1994).

[3] Hofmann K.O., Bucher P. FEBS Lett. 358:153-157(1995).

[4] Sudol M., Chen H.I., Bougeret C., Einbond A., Bork P. FEBS Lett. 369:67-71(1995).

[5] Chen H.I., Sudol M. Proc. Natl. Acad. Sci. U.S.A. 92:7819-7823(1995).

[6] Sudol M., Bork P., Einbond A., Kastury K., Druck T., Negrini M., Huebner K., Lehman D. J. Biol. Chem. 270:14733-14741(1995).

1032. XPA protein signatures. cross-reference(s): XPA_1 PROSITE PS00752; PS00753; XPA_2.

Xeroderma pigmentosum (XP) [1] is a human autosomal recessive disease, characterized by a high incidence of sunlight-induced skin cancer. People's skin cells with this condition are hypersensitive to ultraviolet light, due

to defects in the incision step of DNA excision repair. There are a minimum of seven genetic complementation groups involved in this pathway: XP-A to XP-G. XP-A is the most severe form of the disease and is due to defects in a 30 Kd nuclear protein called XPA (or XPAC) [2].

The sequence of the XPA protein is conserved from higher eukaryotes [3] to yeast (gene RAD14) [4]. XPA is a hydrophilic protein of 247 to 296 amino-acid residues which has a C4-type zinc finger motif in its central section.

Two signature were developed patterns for XPA proteins. The first corresponds to the zinc finger region, the second to a highly conserved region located some 12 residues after the zinc finger region.

Consensus pattern C-x-[DE]-C-x(3)-[LIVMF]-x(1,2)-D-x(2)-L-x(3)-F-x(4)-C-x(2)-C

Consensus pattern [LIVM](2)-T-[KR]-T-E-x-K-x-[DE]-Y-[LIVMF](2)-x-D-x-[DE]

[1] Tanaka K., Wood R.D. Trends Biochem. Sci. 19:83-86(1994).

[2] Miura N., Miyamoto I., Asahina H., Satokata I., Tanaka K., Okada Y. J. Biol. Chem. 266:19786-19789(1991).

[3] Shimamoto T., Kohno K., Tanaka K., Okada Y. Biochem. Biophys. Res. Commun. 181:1231-1237(1991).

[4] Bankmann M., Prakash L., Prakash S. Nature 355:555-558(1992).

1033. YCF9

This family consists of the hypothetical protein product of the YCF9 gene from chloroplasts and cyanobacteria. Number of members: 16

1034. (DUF15)

It is highly conserved between eubacteria and eukaryotes.

Number of members: 30

1035. Luminal portion of Cytochrome b559, alpha (gene psbE) subunit. (cytochr_b559a)

This family is the luminal portion of cytochrome b559 alpha chain, matches to this family should be accompanied by a match to the cytochr_b559 family also. The Prosite pattern pattern matches the transmembrane region of the cytochrome b559 alpha and beta subunits.

5 Number of members: 16

A. Asparaginase 2

10

Asparaginase II (L-asparagine aminohydrolase II) is an extracellular protein that may be associated with the cell wall and whose expression is affected by the availability of nitrogen.

Asparaginase II catalyzes the reaction of L-Asparagine + H₂O = L-Aspartate + NH₃. As many leukemias have high requirements for aspartic acid, asparaginase II proteins are useful as reagents for screening compounds for activity as leukemia chemotherapy products.

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Asparaginase II protein can also be over- or under-expressed to alter amino acid content in plant tissues or to modify nitrogen fixation and/or nitrogen metabolism in plants.

Ref: Bon et al. (1997) Appl Biochem Biotechnol 63-65: 203-12

20

B. Chloroa b-bind

Chlorophyll a-b binding proteins are located in the thylakoid membranes of the chloroplast and bind chlorophyll a and chlorophyll b, thereby triggering a chemical reaction

25

(photosynthesis). These proteins are useful in controlling the rate, efficiency and/or output of photosynthesis. Overexpression of chlorophyll a-b binding proteins is expected to increase the rate of photosynthesis.

Ref: Leutwiler et al. (1986) Nucleic Acids Res 14: 4051-64

30

Brandt et al. (1992) Plant Mol Biol 19: 699-703

C. DMRL synthase

DMRL Synthase (6,7-Dimethyl-8-Ribityllumazine Synthase) catalyzes the last step in riboflavin (Vitamin B₂) synthesis, condensing 5-amino-6-(1'-D)-ribityl-amino-2,4(1H, 3H)-Pyrimidinedione with L-3,4-Dihydroxy-2-Butanone 4-Phosphate producing 6,7-Dimethyl-8-(1-D-Ribityl)Luminazine. The enzyme forms a homopentamer. Engineering of these proteins or those with homologous sequences/structures may allow control of the amounts of vitamin B₂ available in plants and/or accumulation of pigment, as well as altering reactions requiring hydrogen ion carriers/transmitters.

Ref: Garcia-Ramirez et al. (1995) J Biol Chem **270**: 23801-7

D. E1_N

These proteins are ATP-dependent DNA helicases that are required for initiation of viral DNA replication. They form a complex with the viral E2 protein. The E1-E2 complex binds to the replication origin that contains binding sites for both proteins. The majority of sequences known for this group of proteins are from various papillomaviruses, a type of double stranded DNA virus. In plants, the prototype double stranded DNA virus is Cauliflower Mosaic virus (CaMV). Manipulation of these proteins, especially to produce variant proteins that form non-productive complexes, enables production of plants that are resistant to infection by double stranded DNA viruses.

Ref: Yang et al. (1993) PNAS USA **90**: 5086-90

Ustav and Stenlund (1991) EMBO J **10**: 449-57

Callaway et al. (1996) Mol Plant Microbe Interact **9**: 810-8

E. EF1_G

Elongation Factor-1 is composed of four subunits: alpha, beta, delta and gamma. Gamma subunits are presumed to play a role in anchoring the complex to other cellular components. Studies of EF-1 genes in plants suggests that different forms of the EF-1 subunits may be expressed in particular organs or in response to stress. Manipulation of the activity of these proteins, either by altered expression level or by structural mutation, may result in the accumulation of a particular protein in a chosen organ or allow production of particular proteins during stress conditions.

- Ref: Kinzy et al. (1994) NAR 22: 2703-7
Dunn et al. (1993) Plant Mol Biol 23: 221-5
Aguilar et al. (1991) Plant Mol Biol 17: 351-60

F. ENV_polyprotein

This family comprises the envelope or coat proteins known from a number of different retroviruses. In mammalian species, retroviruses are responsible for diseases such as leukemia and HIV. In plants, retroviruses are known in both monocot (e.g. Zeon-1) and dicot (e.g. Arabidopsis and tobacco) species and have been shown to induce mutant alleles at new loci. Engineering of plant ENV proteins may allow mobilization or targeting of endogenous or introduced retroviruses, in essence generating a new method for mutant production, gene tagging and the like.

- Ref: Mamoun et al (1990) J Virol 64: 4180-8
Grandbastien et al. (1989) Nature 337: 376-80
Wright and Voytas (1998) Genetics 149: 703-15

G. Glycosyl_hydr9

Proteins having this domain (previously known as the glycosyl hydrolase family 5 domain) catalyze the endohydrolysis of 1,4- β -D-glucosidic linkages in cellulose. Numerous plant proteins with this domain exist and are expressed in an organ specific manner. They are involved in the fruit ripening process, in cell elongation and plant reproduction. Modulation of the activity of these proteins, either by over- or under-expression or by mutation of the polypeptide, could be used to affect post-harvest physiology (e.g. rate of ripening) or for engineering reproductive sterility.

- Ref: Giorda et al. (1990) Biochemistry 29: 7264-9
Tucker et al. (1988) Plant Physiol 88: 1257-62
Shani et al. (1997) 43: 837-42

H. Glycosyl_hydr14

- 5 The β -amylases (family 14 of glycosyl hydrolases) catalyze the hydrolysis of 1,4- α -glucosidic linkages in polysaccharides and remove successive maltose units from the non-reducing ends of the chains. Mutants of β -amylase in Arabidopsis exhibited altered degradation of starch throughout the diurnal cycle. In addition, the mutant phenotypes indicated that these enzymes not only affect carbohydrate metabolism/catabolism, but also
- 10 influence the amount of pigment stored within particular cells. Manipulation of the β -amylase genes enables control of plant pigmentation (for example, fibre pigment in cotton) as well as carbohydrate synthesis and degradation.

Ref: Zeeman et al. (1998) Plant J 15: 357-65

15 Hirano and Nakamura (1997) Plant Physiol 114: 5675-82

Kitamoto et al. (1988) J Bacteriol 170: 5848-54

I. Glycosyl_hydr15

- 20 Glycosyl hydrolases from family 15 (such as 1,4-Alpha-D-Glucan glucohydrolase,) catalyze the hydrolysis of terminal 1,4-linked alpha-D-glucose residues successively from the non-reducing ends of the chains resulting in the release of β -D-Glucose. In plants these proteins have been tied to the mobilization of the xyloglucan stored in the cotyledonary cell walls.
- 25 Proteins such as these could be varied to affect the rate of plant growth (for example during germination), storage and/or use of glucose and other sugars by plant tissues and alteration of the properties, such as elasticity, of plant cell walls.

Ref: Crombie et al. (1998) Plant J 15: 27-38

30 Hata et al. (1991) Agric Biol Chem 55: 941-9

J. Glycosyl_hydr20

Members of the family 29 glycosyl hydrolases catalyze the hydrolysis of terminal non-reducing N-acetyl-D-hexosamine residues in N-acetyl- β -D-hexosaminides. N-acetyl- β -glucosaminidase belongs to this family and exists in several different forms (consisting of various combinations of alpha and beta chains) depending on the organism. Family 20 glycosyl hydrolases have been implicated in lysosomal storage diseases (such as Sandhoff disease) and glycogen storage disease in humans. These types of proteins are also responsible for the hydrolysis of chitin. In plants, these proteins could be useful in controlling carbohydrate catabolism, thereby influencing the amount of sugars available for storage and/or use in other metabolic pathways. In addition, it is possible that such proteins could be used to engineer an endogenous insect protection mechanism, e.g. by secretion of a chitin-hydrolyzing composition by the plant.

Ref: Graham et al (1988) J Biol Chem 263: 16823-9
O'Dowd et al. (1988) Biochemistry 27: 5216-26

K. HMG box

The HMG box is a novel type of DNA-binding domain found in a diverse group of proteins. Numerous plant proteins contain this domain, such as the HMG1/2-like proteins. The expression of some of these HMG proteins appears to be regulated by circadian rhythms and in a light dependent manner, occurring at higher levels in roots, for example and lower levels in light-grown tissues such as cotyledons. Generally, HMG proteins are thought to influence transcription regulation. In plants, HMGs are believed to have a role in maintaining patterns of circadian-regulated expression for other genes, suggesting that these proteins could be exploited to control growth and development.

Ref: Laudet et al. (1993) Nucleic Acids Res 21: 2493-501
Zheng et al. (1993) Plant Mol Biol 23: 813-23
Grasser et al. (1993) Plant Mol Biol 23: 619-25

L. IL2

Interleukin-2 (IL-2) is produced in mammals by T cells in response to antigenic or mitogenic stimulation and is crucial for proper regulation and functioning of the immune response. IL-2 is capable of stimulating B cells, monocytes, lymphokine-activated killer cells, natural killer cells and glioma cells. Plant extracts have also been shown to stimulate the immune system (for example, mistletoe therapy for human cancer). It is known that IL-2 is involved in feedback inhibition pathways that impact the inflammatory response as well as the growth inhibition of tumor reactive T cells. Plant proteins containing IL-2-like sequences are useful as immunity-based therapeutics, acting in a manner similar to IL-2 in mammals.

- 10 Ref: Heike et al. (1997) Scand J Immunol 45: 221-6
 Ariel et al. (1998) J Immunol 161: 2465-72
 Schink (1997) Anticancer Drugs 8 Suppl 1: S47-51

M. Oxidored_FMN

- 15 NADPH dehydrogenases catalyze the reaction $\text{NADPH} + \text{acceptor} = \text{NADP}(+) + \text{reduced acceptor}$. One member of this family is yeast "old yellow enzyme" (OYE) and is thought to be involved in oxylipin metabolism. A second yeast family member is a protein that binds estrogen binding protein (EBP) in addition to exhibiting oxidoreductase activity. An Arabidopsis homolog to OYE has been described and estrogen binding proteins in plants have been reported. Plant proteins from this class have the potential to be used to modify lipid metabolism/catabolism. These proteins may also have use as therapeutics for breast and prostate cancer, and other abnormal growth in steroid-sensitive tissues.
- 20 Ref: Baker et al. (1998) Proc Soc Exp Biol Med 217: 317-21
 Schaller and Weiler (1997) J Biol Chem 272: 28066-72
 Mandani et al. (1994) PNAS USA 91: 922-6

N. Oxidored_q2

- 30 The NADH-plastoquinone oxidoreductases catalyze the reaction $\text{NADH} + \text{plastoquinone} = \text{NAD}(+) + \text{plastoquinol}$. In plants these reactions occur in the chloroplast and are believed to participate in a chloroplast respiratory system. Here, the NDH complex is postulated to act as

a valve to remove excess reduction equivalents in the chloroplasts. Manipulation of these proteins may improve the rate or efficiency of photosynthesis.

Ref: Burrows et al. (1998) EMBO J 17: 868-76

Kofer et al (1998) Mol Gen Genet 258: 166-73

Maier et al. (1995) J Mol Biol 251: 614-28

O. PABP

Polyadenylate binding proteins bind the poly (A) tail of mRNA. Plants, as exemplified by Arabidopsis, contain numerous PABP genes that are expressed in an organ-specific manner. For example, PABP2 is functional in roots and shoots, while PABP5 is expressed predominantly in immature flowers. The PABP proteins are implicated in numerous aspects of postranscriptional regulation including mRNA turnover and translational initiation.

Control of activity of PABP proteins provides the ability to control the expression of various genes in particular organs during development.

Ref: Hilson et al (1993) Plant Physiol 103: 525-33

Belostotsky and Meagher (1993) PNAS USA 90: 6686-90

P. Parvo coat

Parvoviruses are linear single-stranded DNA viruses that are encapsulated by three capsid proteins. Plants are susceptible to infection by single stranded DNA viruses such as Maize streak virus (MSV) and various Gemini viruses. The coat proteins in these plant viruses are critical to the virus life cycle within the plant. For example, the coat protein of MSV is thought to be involved in intra- and inter-cellular movement within the plant. Engineering of proteins having similarity to parvoviral coat proteins, especially to produce proteins that interfere with maturation of the virus particle, enables the production of plants having better resistance to natural plant single-stranded DNA viruses.

Ref: Liu et al. (1997) J Gen Virol 78: 1265-70

Rohde et al. (1990) Virology 176: 648-51

O. Pkinase_C

Plant serine/threonine protein kinases possessing this domain are expressed in all tissues and are known to undergo serine-specific autophosphorylation and specifically phosphorylate two ribosomal proteins, P14 and P16. During development, these proteins predominate during high metabolic activity in growing buds, root tips, leaf margins and germinating seeds. They are thought to be involved in the control of plant growth and development. In addition, two genes encoding proteins from this family have been described that help plant cells adapt during cold or high salt stresses. Consequently, engineering Pkinase C proteins provides a way to control general growth/development of the plant as well as a means to provide endogenous protection against environmental stresses.

Ref: Zhang et al. (1994) J Biol Chem 269: 17586-92

Mizoguchi et al. (1995) FEBS Lett 358: 199-204

R. REV

The REV proteins act post-transcriptionally to relieve negative repression of GAG and ENV production in retroviruses such as Human Immunodeficiency Virus type I (HIV-1). Plants contain retrovirus-like viruses such as pararetroviruses and retrotransposons (i.e. transposons having long terminal repeats). Plant retrotransposons in particular have been used to create mutations at various loci, thereby permitting gene isolation, gene tagging and the like. Manipulation of plant REV proteins enables control of transposition frequencies of corresponding transposable elements and provides a new tool for genetic engineering of plants.

Ref: Sodroski et al. (1986) Nature 321: 412-7

Franchini et al. (1989) PNAS USA 86: 2433-7

Marquet et al. (1995) 77: 113-24

Grandbastien et al. (1989) Nature 337: 376-80

Wright and Voytas (1998) Genetics 149: 703-15

S. RuBisCo small

Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo) catalyzes the initial step in the C3 photosynthetic carbon reduction cycle, adding carbon dioxide to D-ribulose 1,5-bisphosphate to form two molecules of 3-phospho-D-glycerate. RuBisCo is comprised of two subunits, one large which is synthesized in the chloroplast, and one small which is synthesized in the cytoplasm and then transported in to the chloroplast. The expression of the small subunit of RuBisCo is light regulated. Manipulation of these proteins could increase the efficiency of photosynthesis or allow alterations in developmental timing.

Ref: Giuliano et al. (1988) PNAS USA 85: 7089-93

Dedonder et al. (1993) Plant Physiol 101: 801-8

T. Sialyltransf

Members of the CMP-N-acetylneuraminate- β -galactosamide- α -2,3-sialyltransferase family catalyze the following reaction:

CMP-N-acetylneuraminate + β -D-galactosyl-1,3-N-acetyl- α -D-galactosaminyl-R = CMP + α -N-acetylneuraminyl-2,3- β -D-galactosyl-1,3-N-acetyl- α -D-galactosaminyl-R. These proteins are thought to be responsible for the synthesis of the sequence neurac- α -2,3-gal- β -1,3-galnac- found on sugar chains)-linked to threonine or serine and also as a terminal sequence on certain gangliosides in mammalian cells. In plants, glycosyltransferases in the Golgi apparatus synthesize cell wall polysaccharides and elaborate the complex glycans of glycoproteins. Engineering of plant sialyltransferases allows targeting of proteins to particular cellular locations or enables the making of changes in cell wall structure.

Ref: Wee et al. (1998) Plant Cell 10: 1759-68

Lee et al. (1994) J Biol Chem 269: 10028-33

Kitagawa and Paulson (1994) J Biol Chem 269: 1394-401

U. Signal

Many plant proteins in this family contain sequences similar to those found in both components of the prokaryotic family of signal transducers known as the two-component systems. This suggests that activation may require a transfer of a phosphate group between

the transmitter domain and the receiver domain. One family member in Arabidopsis appears to be involved in ethylene (a plant hormone) signal transduction. Other proteins in this family appear to be involved in the regulation of gene transcription under conditions of environmental stress. Signal proteins can be exploited to affect plant growth and development and/or control plant responses to stress conditions such as cold, nutrient availability, etc.

Ref: Chang et al. (1993) Science 262: 539-44

Nagaya et al. (1993) Gene 131: 119-124

Gottfert et al. (1990) PNAS USA 87: 2680-4

V. vMSA

vMSA proteins are major surface antigens presenting on the envelope of various retroviruses. Surface antigens of retroviruses are often involved in tropism of the virus.

Plants contain retrovirus-like viruses such as pararetroviruses and retrotransposons (i.e. transposons having long terminal repeats). Plant retrotransposons in particular have been used to create mutants at various loci, thereby permitting gene isolation, gene tagging and the like. Manipulation of plant vMSA proteins enables control of tropism of plant retroviruses that might be used for genetic engineering tools, thus enabling targeting of the virus to particular species and/or tissues of plants.

Ref: Okamoto et al. (1988) J Gen Virol 69: 2575-83

Grandbastien et al. (1989) Nature 337: 376-80

Wright and Voytas (1998) Genetics 149: 703-15

W. zf-CCCH

This family of proteins is defined by having two CX(8)CX(5)CX(3)H-type zinc finger domains. These proteins cover a broad range of functions. For example, the COP1 protein acts as a repressor of photomorphogenesis in darkness; light stimuli abolish this suppressive action. In addition, COP1 protein can function as a negative transcriptional regulator capable of direct interaction with components of the G-protein signaling pathway. As a second example, a zf-CCCH protein identified in Arabidopsis appears to be involved in the resistance to DNA damage induced by UV light and chemical DNA-damaging agents.

Overexpression of this class of proteins permits production of plants that are better suited to adverse environments. Manipulation of expression of zf-CCCH proteins functioning as transcriptional regulators, such as COP1, enables manipulation of some signal transduction pathways.

Ref: Pang et al. (1993) Nucleic Acids Res 21: 1647-53
Deng et al. (1992) Cell 71: 791-801

X. zf-RanBP

Proteins falling within this category contain many X-X-F-G and X-F-X-F-G repeats, and may contain RANBP1-like or PPIase domains. Plant proteins having domains similar to these include PAS1 and GMSTI. PAS1 has been shown to have dramatic developmental affects that appear to be correlated with both cell division and cell wall elongation. GMSTI has high identity to the yeast STI stress-inducible gene and has been shown to be heat inducible. Proteins such as these may be useful for controlling growth and form of development.

Ref: Vittorioso et al. (1998) Mol Cell Biol 18: 3034-43
Hernandez Torres et al. (1995) 27: 1221-6

Y. Peptidase M48

Proteins belonging to this peptidase family are metalloproteases that bind zinc as a cofactor and are located in the membranes of the endoplasmic reticulum. They function in NH₂-terminal proteolytic processing, as shown for the yeast STE24 gene product. This gene is required for the correct processing of α -factor, a yeast pheromone. Family M48 peptidases also appear to be required for some prenylation reactions, mediating COOH-terminal CAAX processing. Prenylation reactions are believed to be involved in the regulation of protein-protein and protein-membrane interactions. As an example, RAS GTPase activity is regulated in part by localization to the inner side of the plasma membrane upon prenylation. In plants, proteins from this family could be involved in pollen-stigma interactions such as those mediating self-pollination vs. outcrossing, or could be members of several secondary metabolism pathways.

Ref: Fujimura-Kamada et al. (1997) J Cell Biol. 136: 271-85. Tam et al. (1998) J Cell Biol. 142: 635-49.

Z. DNA Pol Viral N

The DNA pol Viral N domain is located at the N-terminal region of DNA polymerase isolated from several retrovirus viruses such as the Cauliflower Mosaic Virus. The domain motif has also been found in numerous other species from humans to cyanobacteria. In these organisms, this motif seems to be associated with two types of sequences; retrotransposons and mitochondrial genes. In the mitochondrial sequences this domain is potentially involved in the self-splicing conducted by group II introns. Various manipulations of this gene in plants allows control of the numerous retrotransposons endogenous to plant genomes or allows engineering of mitochondrial function, especially to increase efficiency of energy utilization by cells.

REF: Chapdelaine and Bonen (1991) Cell 65: 465-72
 Ferat and Miche (1993) Nature 364: 358-61
 Wilson et al. (1994) 368: 32-8
 Cambareri et al. (1994) 242: 658-65
 Gaardner et al. (1981) NAR 9: 2871-2888
 Cummings et al. (1990) Curr Genet 17: 375-402
 Hattori et al. (1986) Nature 321: 625-8

Aa. Calpain_inhib

This domain is found in calpastatin, an inhibitor protein specific for calpain. Calpain is a non-lysosomal calcium-dependent intracellular protease that appears to be involved in the dynamic changes of the cytoskeleton, especially actin-related structures, during early *Drosophila* embryogenesis [1]. Calpastatins co-exist in cells with calpains and the subcellular distribution of calpastatin is thought to be important to calpain regulation [2]. In plants calpains and calpastatins could be involved in embryogenesis and non-embryogenic organ reiteration. Mutations occurring in calpain inhibitor repeat domains would produce developmental abnormalities such as abnormal leaf, root or flower development.

Refs

- 1 Emori Y and Saigo K (1994) J Biol Chem 269: 25137-42.
- 2 Mellgren RL, Lane RD, Mericle MT (1989) Biochim Biophys Acta 999: 71-77.

Ab. chorismate_bind

5 Chorismate binding domains are present in plant anthranilate synthase (AS) genes. AS genes catalyze the first step in the biosynthesis of tryptophan by converting chorismate and L-glutamine to anthranilate, pyruvate and L-glutamate. Some of these genes are involved in feedback inhibition by tryptophan [1] while some are feedback insensitive [2]. In Arabidopsis, two AS genes have overlapping, but different distributions. One of these AS
10 genes is induced by wounding and bacterial pathogen infiltration [1]. Mutations in the chorismate binding domain would affect the production of tryptophan and could influence the plant's defense system. AS gene products can be used for *in vitro* synthesis of tryptophan and tryptophan derivatives.

15 Refs

- 1 Niyogi KK, Fink GR (1992) Plant Cell 4: 721-33.
- 2 Song HS, Brotherton JE, Gonzales RA, Wilholm JM (1998) Plant Physiol 117:533-43.

20 Ac. late protein_L2

Papillomaviruses are encapsulated double stranded DNA viruses. Plants are susceptible to infection by double stranded DNA viruses such as Cauliflower Mosaic virus (CaMV). The coat proteins in these plant viruses are critical to the virus life cycle within the plant. For example, the coat protein of CaMV is thought to be involved in intra- and inter-cellular
25 movement within the plant [1]. Engineering of proteins having similarity to papillomavirus coat proteins may enable the production of plants having better resistance to natural plant double stranded DNA viruses.

Refs

- 30 1 Thompson SR, Melcher U (1993) J Gen Virol 74: 1141-8.

Ad. Peptidase_M41

Proteins belonging to this peptidase family are metalloproteases that bind zinc as a cofactor and are integral membrane proteins. They seem to be involved in the degradation of carboxy-

terminal-tagged cytoplasmic proteins. In plants, these proteins are located in the thylakoid membranes of the chloroplasts, their expression is light regulated and they are thought to be involved in degradation of soluble stromal proteins and turn-over of thylakoid proteins [1].

Manipulation of expression and structure of these proteins would have effects on the

efficiency of photosynthesis and the development of chloroplasts.

Refs

- 1 Lindahl M, Tabak s, Cseke L, Pichersky E, Andersson B, Adam Z (1996) J Biol Chem 271: 29329-34.

Ae. UPF0051

There is some evidence that, in plants, proteins in this family are involved in ATP synthesis in chloroplasts [1, 2]. Mutations in these proteins or altering their expression would affect the efficiency of photosynthesis and energy production.

Refs

- 1 Kostrzewa M, Zetsche K (1992) J Mol Biol 227: 961-70.
- 2 Kostrzewa M, Zetsche K (1993) Plant Mol Biol 23: 67-76

Af. E7

Papillomaviruses are encapsulated double stranded DNA viruses. The Papillomavirus early protein 7 (E7) is known as a potent immortalizing and transforming agent. Transformation by E7 is thought to be mediated by the physical association of E7 with cellular proteins regulating entry into the cell cycle [1]. The result is entry into the cell cycle and suppression of terminal differentiation in mammalian cells. Thus, engineering of proteins having similarity to papillomavirus E7 protein enables the production of plants having altered cellular proliferation characteristics and possibly altered morphology. For example, overexpression of E7-like proteins would be expected to result in proliferation of cells of the tissue in which the E7 protein is expressed, perhaps with suppression of differentiation events. Thus, for example, overexpression of E7-like proteins in meristem cells can result in taller plants and suppression of leafing and/or flowering.

Refs

- 1 Zwerschke W, Jansen-Durr P Adv Cancer Res 2000;78:1-29

Ag. Peptidase U7

This protein is known to be an integral membrane protein in the cyanobacterium *Synechocystis* where it functions to digest cleaved signal peptides [1]. This activity is necessary to maintain proper secretion of mature proteins across the membrane. In higher plants this protein may be present in the plastid or chloroplast membranes where it would function by enabling protein movement into and out of the chloroplasts. Mutations in this protein would be expected to affect the development of plastids, including chloroplasts, or alter the energy transfer system within the chloroplasts, thereby affecting growth and development.

Refs

- 1 Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirose M, Sugita M, Sasamoto S, Kimura T, Hosouchi T, Matsuno A, Muraki A, Nakazaki N, Naruo K, Okumura S, Shimpo S, Takeuchi C, Wada T, Watanabe A, Yamada M, Yasuda M, Tabata S (1996) *DNA Res* 3:109-36.

Ah. 5'-3' Exonuclease

The 5'-3' exonuclease domain is one found in bacterial DNA polymerases I and in yeast DNA repair enzymes such as Exonuclease I. Yeast Exo I is involved in mitotic recombination and also includes a domain that interacts with the mismatch repair protein MSH2. The 5'-3' exonuclease domain is also present in XPG DNA repair enzymes in humans and in yeast RAD9 protein. Defects in XPG proteins result in Xeroderma Pigmentosum. Thus defects in 5'-3' exonuclease domain-containing proteins in plants are expected to lead to defects in DNA repair and corresponding high spontaneous and inducible mutation rates. Consensus sequence:

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IMKKKLLLV DGSSLAFFALPPLTNSAGEPTNAVYGLKMLIKLIEQEOPTHIAVV
FDAQAKTFRHELYEGYKAGRAP
TPDELREIQIPLIKELLDALGIPLLEVAGYEADDVIGTLAKLAEKEGYEVLIVTGDRDLL
QLVSDHVTVIITKKGIAEFTL
FTPEAVIEKYGLTPEQIIDYKALMGDSSDNIPGVKGIGECTAAKLLQEYGSLEGYIANL
DKLKGKKLREKLLAHKEDAKL
SRDLATIKTDVPLDLTLDDLRLPDPDRDALDLLFDE

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Ref:

- Fiorentini P. et al. RT. Mol. Cell. Biol. 17:2764-2773(1997).
Tishkoff et al. Cancer Res. 0:0-0(1998).
Macinnes M.A. et al. Mol. Cell. Biol. 13:6393-6402(1993).

AA. Activities of Polypeptides Comprising Signal Peptides

Polypeptides comprising signal peptides are a family of proteins that are typically targeted to (1) a particular organelle or intracellular compartment, (2) interact with a particular molecule or (3) for secretion outside of a host cell. Example of polypeptides comprising signal peptides include, without limitation, secreted proteins, soluble proteins, receptors, proteins retained in the ER, etc.

These proteins comprising signal peptides are useful to modulate ligand-receptor interactions, cell-to-cell communication, signal transduction, intracellular communication, and activities and/or chemical cascades that take part in an organism outside or within of any particular cell.

One class of such proteins are soluble proteins which are transported out of the cell. These proteins can act as ligands that bind to receptor to trigger signal transduction or to permit communication between cells.

Another class is receptor proteins which also comprise a retention domain that lodges the receptor protein in the membrane when the cell transports the receptor to the surface of the cell. Like the soluble ligands, receptors can also modulate signal transduction and communication between cells.

In addition the signal peptide itself can serve as a ligand for some receptors. An example is the interaction of the ER targeting signal peptide with the signal recognition particle (SRP). Here, the SRP binds to the signal peptide, halting translation, and the resulting SRP complex then binds to docking proteins located on the surface of the ER, prompting transfer of the protein into the ER.

A description of signal peptide residue composition is described below in Subsection

IV.C.1.

III. Methods of Modulating Polypeptide Production

It is contemplated that polynucleotides of the invention can be incorporated into a host cell or in-vitro system to modulate polypeptide production. For instance, the SDFs prepared as described herein can be used to prepare expression cassettes useful in a number of techniques for suppressing or enhancing expression.

An example are polynucleotides comprising sequences to be transcribed, such as coding sequences, of the present invention can be inserted into nucleic acid constructs to modulate polypeptide production. Typically, such sequences to be transcribed are heterologous to at least one element of the nucleic acid construct to generate a chimeric gene or construct.

Another example of useful polynucleotides are nucleic acid molecules comprising regulatory sequences of the present invention. Chimeric genes or constructs can be generated when the regulatory sequences of the invention linked to heterologous sequences in a vector construct. Within the scope of invention are such chimeric gene and/or constructs.

Also within the scope of the invention are nucleic acid molecules, whereof at least a part or fragment of these DNA molecules are presented in TABLE 1 of the present application, and wherein the coding sequence is under the control of its own promoter and/or its own regulatory elements. Such molecules are useful for transforming the genome of a host cell or an organism regenerated from said host cell for modulating polypeptide production.

Additionally, a vector capable of producing the oligonucleotide can be inserted into the host cell to deliver the oligonucleotide.

More detailed description of components to be included in vector constructs are described both above and below.

Whether the chimeric vectors or native nucleic acids are utilized, such polynucleotides can be incorporated into a host cell to modulate polypeptide production. Native genes and/or nucleic acid molecules can be effective when exogenous to the host cell.

Methods of modulating polypeptide expression includes, without limitation:

Suppression methods, such as

Antisense

Ribozymes

Co-suppression

Insertion of Sequences into the Gene to be Modulated

Regulatory Sequence Modulation.

as well as Methods for Enhancing Production, such as
Insertion of Exogenous Sequences; and
Regulatory Sequence Modulation.

III.A. Suppression

Expression cassettes of the invention can be used to suppress expression of endogenous genes which comprise the SDF sequence. Inhibiting expression can be useful, for instance, to tailor the ripening characteristics of a fruit (Oeller et al., *Science* 254:437 (1991)) or to influence seed size, (WO98/07842) or to provoke cell ablation (Mariani et al., *Nature* 357: 384-387 (1992)).

As described above, a number of methods can be used to inhibit gene expression in plants, such as antisense, ribozyme, introduction of exogenous genes into a host cell, insertion of a polynucleotide sequence into the coding sequence and/or the promoter of the endogenous gene of interest, and the like.

III.A.1. Antisense

An expression cassette as described above can be transformed into host cell or plant to produce an antisense strand of RNA. For plant cells, antisense RNA inhibits gene expression by preventing the accumulation of mRNA which encodes the enzyme of interest, *see*, e.g., Sheehy et al., *Proc. Nat. Acad. Sci. USA*, 85:8805 (1988), and Hiatt et al., U.S. Patent No. 4,801,340.

III.A.2. Ribozymes

Similarly, ribozyme constructs can be transformed into a plant to cleave mRNA and down-regulate translation.

III.A.3. Co-Suppression

Another method of suppression is by introducing an exogenous copy of the gene to be suppressed. Introduction of expression cassettes in which a nucleic acid is configured in the sense orientation with respect to the promoter has been shown to prevent the accumulation of mRNA. A detailed description of this method is described above.

III.A.4. Insertion of Sequences into the Gene to be Modulated

Yet another means of suppressing gene expression is to insert a polynucleotide into the gene of interest to disrupt transcription or translation of the gene.

Homologous recombination could be used to target a polynucleotide insert to a gene using the Cre-Lox system (A.C. Vergunst et al., *Nucleic Acids Res.* **26**:2729 (1998), A.C. Vergunst et al., *Plant Mol. Biol.* **38**:393 (1998), H. Albert et al., *Plant J.* **7**:649 (1995)).

In addition, random insertion of polynucleotides into a host cell genome can also be used to disrupt the gene of interest. Azpiroz-Leehan et al., *Trends in Genetics* **13**:152 (1997). In this method, screening for clones from a library containing random insertions is preferred for identifying those that have polynucleotides inserted into the gene of interest. Such screening can be performed using probes and/or primers described above based on sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto. The screening can also be performed by selecting clones or any transgenic plants having a desired phenotype.

III.A.5. Regulatory Sequence Modulation

The SDFs described in Table 1, and fragments thereof are examples of nucleotides of the invention that contain regulatory sequences that can be used to suppress or inactivate transcription and/or translation from a gene of interest as discussed in I.C.5.

III.A.6. Genes Comprising Dominant-Negative Mutations

When suppression of production of the endogenous, native protein is desired it is often helpful to express a gene comprising a dominant negative mutation. Production of protein variants produced from genes comprising dominant negative mutations is a useful tool for research. Genes comprising dominant negative mutations can produce a variant polypeptide which is capable of competing with the native polypeptide, but which does not produce the native result. Consequently, over expression of genes comprising these mutations can titrate out an undesired activity of the native protein. For example, The product from a gene comprising a dominant negative mutation of a receptor can be used to constitutively activate or suppress a signal transduction cascade, allowing examination of the phenotype and thus the trait(s) controlled by that receptor and pathway. Alternatively, the protein arising from the gene comprising a dominant-negative mutation can be an inactive enzyme still capable of binding to the same substrate as the native protein and therefore competes with such native protein.

Products from genes comprising dominant-negative mutations can also act upon the native protein itself to prevent activity. For example, the native protein may be active only as a homo-multimer or as one subunit of a hetero-multimer. Incorporation of an inactive subunit into the multimer with native subunit(s) can inhibit activity.

Thus, gene function can be modulated in host cells of interest by insertion into these cells vector constructs comprising a gene comprising a dominant-negative mutation.

III.B. Enhanced Expression

Enhanced expression of a gene of interest in a host cell can be accomplished by either (1) insertion of an exogenous gene; or (2) promoter modulation.

III.B.1. Insertion of an Exogenous Gene

Insertion of an expression construct encoding an exogenous gene can boost the number of gene copies expressed in a host cell.

Such expression constructs can comprise genes that either encode the native protein that is of interest or that encode a variant that exhibits enhanced activity as compared to the native protein. Such genes encoding proteins of interest can be constructed from the sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto.

Such an exogenous gene can include either a constitutive promoter permitting expression in any cell in a host organism or a promoter that directs transcription only in particular cells or times during a host cell life cycle or in response to environmental stimuli.

III.B.2. Regulatory Sequence Modulation

The SDFs of Table 1, and fragments thereof, contain regulatory sequences that can be used to enhance expression of a gene of interest. For example, some of these sequences contain useful enhancer elements. In some cases, duplication of enhancer elements or insertion of exogenous enhancer elements will increase expression of a desired gene from a particular promoter. As other examples, all II promoters require binding of a regulatory protein to be activated, while some promoters may need a protein that signals a promoter binding protein to expose a polymerase binding site. In either case, over-production of such proteins can be used to enhance expression of a gene of interest by increasing the activation time of the promoter.

Such regulatory proteins are encoded by some of the sequences in TABLE 1, fragments thereof, and substantially similar sequences thereto.

Coding sequences for these proteins can be constructed as described above.

IV. Gene Constructs and Vector Construction

To use isolated SDFs of the present invention or a combination of them or parts and/or mutants and/or fusions of said SDFs in the above techniques, recombinant DNA vectors which
5 comprise said SDFs and are suitable for transformation of cells, such as plant cells, are usually prepared. The SDF construct can be made using standard recombinant DNA techniques (Sambrook et al. 1989) and can be introduced to the species of interest by *Agrobacterium*-mediated transformation or by other means of transformation (e.g., particle gun bombardment) as referenced below.

10 The vector backbone can be any of those typical in the art such as plasmids, viruses, artificial chromosomes, BACs, YACs and PACs and vectors of the sort described by

(a) **BAC:** Shizuya et al., Proc. Natl. Acad. Sci. USA 89: 8794-8797 (1992);
Hamilton et al., Proc. Natl. Acad. Sci. USA 93: 9975-9979 (1996);

(b) **YAC:** Burke et al., Science 236:806-812 (1987);.

15 (c) **PAC:** Sternberg N. et al., Proc Natl Acad Sci U S A. Jan;87(1):103-7 (1990);

(d) **Bacteria-Yeast Shuttle Vectors:** Bradshaw et al., Nucl Acids Res 23: 4850-4856 (1995);

(e) **Lambda Phage Vectors:** Replacement Vector, e.g.,
Frischauf et al., J. Mol Biol 170: 827-842 (1983); or Insertion vector, e.g.,

20 Huynh et al., In: Glover NM (ed) DNA Cloning: A practical Approach, Vol.1 Oxford: IRL Press (1985);

(f) **T-DNA gene fusion vectors:** Walden et al., Mol Cell Biol 1: 175-194 (1990);
and

(g) **Plasmid vectors:** Sambrook et al., infra.

25 Typically, a vector will comprise the exogenous gene, which in its turn comprises an SDF of the present invention to be introduced into the genome of a host cell, and which gene may be an antisense construct, a ribozyme construct chimera, or a coding sequence with any desired transcriptional and/or translational regulatory sequences, such as promoters, UTRs, and 3' end termination sequences. Vectors of the invention can also include origins of
30 replication, scaffold attachment regions (SARs), markers, homologous sequences, introns, etc.

A DNA sequence coding for the desired polypeptide, for example a cDNA sequence encoding a full length protein, will preferably be combined with transcriptional and translational

initiation regulatory sequences which will direct the transcription of the sequence from the gene in the intended tissues of the transformed plant.

For example, for over-expression, a plant promoter fragment may be employed that will direct transcription of the gene in all tissues of a regenerated plant. Alternatively, the plant promoter may direct transcription of an SDF of the invention in a specific tissue (tissue-specific promoters) or may be otherwise under more precise environmental control (inducible promoters).

If proper polypeptide production is desired, a polyadenylation region at the 3'-end of the coding region is typically included. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA.

The vector comprising the sequences from genes or SDF or the invention may comprise a marker gene that confers a selectable phenotype on plant cells. The vector can include promoter and coding sequence, for instance. For example, the marker may encode biocide resistance, particularly antibiotic resistance, such as resistance to kanamycin, G418, bleomycin, hygromycin, or herbicide resistance, such as resistance to chlorosulfuron or phosphinotricin.

IV.A. Coding Sequences

Generally, the sequence in the transformation vector and to be introduced into the genome of the host cell does not need to be absolutely identical to an SDF of the present invention. Also, it is not necessary for it to be full length, relative to either the primary transcription product or fully processed mRNA. Furthermore, the introduced sequence need not have the same intron or exon pattern as a native gene. Also, heterologous non-coding segments can be incorporated into the coding sequence without changing the desired amino acid sequence of the polypeptide to be produced.

IV.B. Promoters

As explained above, introducing an exogenous SDF from the same species or an orthologous SDF from another species can modulate the expression of a native gene corresponding to that SDF of interest. Such an SDF construct can be under the control of either a constitutive promoter or a highly regulated inducible promoter (e.g., a copper inducible promoter). The promoter of interest can initially be either endogenous or heterologous to the species in question. When re-introduced into the genome of said species, such promoter becomes exogenous to said species. Over-expression of an SDF transgene can

lead to co-suppression of the homologous endogenous sequence thereby creating some alterations in the phenotypes of the transformed species as demonstrated by similar analysis of the chalcone synthase gene (Napoli et al., *Plant Cell* 2:279 (1990) and van der Krol et al., *Plant Cell* 2:291 (1990)). If an SDF is found to encode a protein with desirable characteristics, its over-production can be controlled so that its accumulation can be manipulated in an organ- or tissue-specific manner utilizing a promoter having such specificity.

Likewise, if the promoter of an SDF (or an SDF that includes a promoter) is found to be tissue-specific or developmentally regulated, such a promoter can be utilized to drive or facilitate the transcription of a specific gene of interest (e.g., seed storage protein or root-specific protein). Thus, the level of accumulation of a particular protein can be manipulated or its spatial localization in an organ- or tissue- specific manner can be altered.

IV. C Signal Peptides

SDFs of the present invention containing signal peptides are indicated in Table 1. In some cases it may be desirable for the protein encoded by an introduced exogenous or orthologous SDF to be targeted (1) to a particular organelle intracellular compartment, (2) to interact with a particular molecule such as a membrane molecule or (3) for secretion outside of the cell harboring the introduced SDF. This will be accomplished using a signal peptide.

Signal peptides direct protein targeting, are involved in ligand-receptor interactions and act in cell to cell communication. Many proteins, especially soluble proteins, contain a signal peptide that targets the protein to one of several different intracellular compartments. In plants, these compartments include, but are not limited to, the endoplasmic reticulum (ER), mitochondria, plastids (such as chloroplasts), the vacuole, the Golgi apparatus, protein storage vesicles (PSV) and, in general, membranes. Some signal peptide sequences are conserved, such as the Asn-Pro-Ile-Arg amino acid motif found in the N-terminal propeptide signal that targets proteins to the vacuole (Marty (1999) *The Plant Cell* 11: 587-599). Other signal peptides do not have a consensus sequence *per se*, but are largely composed of hydrophobic amino acids, such as those signal peptides targeting proteins to the ER (Vitale and Denecke (1999) *The Plant Cell* 11: 615-628). Still others do not appear to contain either a consensus sequence or an identified common secondary sequence, for instance the chloroplast stromal targeting signal peptides (Keegstra and Cline (1999) *The Plant Cell* 11: 557-570). Furthermore, some targeting peptides are bipartite, directing proteins first to an organelle and then to a membrane within the organelle (e.g. within the thylakoid lumen of the

chloroplast; see Keegstra and Cline (1999) *The Plant Cell* 11: 557-570). In addition to the diversity in sequence and secondary structure, placement of the signal peptide is also varied. Proteins destined for the vacuole, for example, have targeting signal peptides found at the N-terminus, at the C-terminus and at a surface location in mature, folded proteins. Signal peptides also serve as ligands for some receptors.

These characteristics of signal proteins can be used to more tightly control the phenotypic expression of introduced SDFs. In particular, associating the appropriate signal sequence with a specific SDF can allow sequestering of the protein in specific organelles (plastids, as an example), secretion outside of the cell, targeting interaction with particular receptors, etc. Hence, the inclusion of signal proteins in constructs involving the SDFs of the invention increases the range of manipulation of SDF phenotypic expression. The nucleotide sequence of the signal peptide can be isolated from characterized genes using common molecular biological techniques or can be synthesized in vitro.

In addition, the native signal peptide sequences, both amino acid and nucleotide, described in Table 1 can be used to modulate polypeptide transport. Further variants of the native signal peptides described in Table 1 are contemplated. Insertions, deletions, or substitutions can be made. Such variants will retain at least one of the functions of the native signal peptide as well as exhibiting some degree of sequence identity to the native sequence.

Also, fragments of the signal peptides of the invention are useful and can be fused with other signal peptides of interest to modulate transport of a polypeptide.

V. Transformation Techniques

A wide range of techniques for inserting exogenous polynucleotides are known for a number of host cells, including, without limitation, bacterial, yeast, mammalian, insect and plant cells.

Techniques for transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. See, e.g. Weising et al., *Ann. Rev. Genet.* 22:421 (1988); and Christou, *Euphytica*, v. 85, n.1-3:13-27, (1995).

DNA constructs of the invention may be introduced into the genome of the desired plant host by a variety of conventional techniques. For example, the DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts, or the DNA constructs can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. Alternatively, the DNA constructs may be combined with suitable T-DNA flanking regions and

introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria (McCormac et al., *Mol. Biotechnol.* 8:199 (1997); Hamilton, *Gene* 200:107 (1997); Salomon et al. *EMBO J.* 3:141

(1984); Herrera-Estrella et al. *EMBO J.* 2:987 (1983).

Microinjection techniques are known in the art and well described in the scientific and patent literature. The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski et al. *EMBO J.* 3:2717 (1984). Electroporation techniques are described in Fromm et al. *Proc. Natl. Acad. Sci. USA* 82:5824 (1985). Ballistic transformation techniques are described in Klein et al. *Nature* 327:773 (1987). *Agrobacterium tumefaciens*-mediated transformation techniques, including disarming and use of binary or co-integrate vectors, are well described in the scientific literature. See, for example Hamilton, *CM., Gene* 200:107 (1997); Müller et al. *Mol. Gen. Genet.* 207:171 (1987); Komari et al. *Plant J.* 10:165 (1996); Venkateswarlu et al. *Biotechnology* 9:1103 (1991) and Gleave, *AP., Plant Mol. Biol.* 20:1203 (1992); Graves and Goldman, *Plant Mol. Biol.* 7:34 (1986) and Gould et al., *Plant Physiology* 95:426 (1991).

Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant that possesses the transformed genotype and thus the desired phenotype such as seedlessness. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the desired nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans et al., *Protoplasts Isolation and Culture in Handbook of Plant Cell Culture*, pp. 124-176, MacMillan Publishing Company, New York, 1983; and Binding, *Regeneration of Plants, Plant Protoplasts*, pp. 21-73, CRC Press, Boca Raton, 1988. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al. *Ann. Rev. of Plant Phys.* 38:467 (1987). Regeneration of monocots (rice) is described by Hosoyama et al. (*Biosci. Biotechnol. Biochem.* 58:1500 (1994)) and by Ghosh et al. (*J. Biotechnol.* 32:1 (1994)). The nucleic acids of the invention can be used to confer desired traits on essentially any plant.

Thus, the invention has use over a broad range of plants, including species from the genera *Anacardium*, *Arachis*, *Asparagus*, *Atropa*, *Avena*, *Brassica*, *Citrus*, *Citrullus*, *Capsicum*, *Carthamus*, *Cocos*, *Coffea*, *Cucumis*, *Cucurbita*, *Daucus*, *Elaeis*, *Fragaria*, *Glycine*, *Gossypium*, *Helianthus*, *Heterocallis*, *Hordeum*, *Hyoscyamus*, *Lactuca*, *Linum*, *Lolium*, *Lupinus*,

Lycopersicon, Malus, Manihot, Majorana, Medicago, Nicotiana, Olea, Oryza, Panieum, Pannasetum, Persea, Phaseolus, Pistachia, Pisum, Pyrus, Prunus, Raphanus, Ricinus, Secale, Senecio, Sinapis, Solanum, Sorghum, Theobromus, Trigonella, Triticum, Vicia, Vitis, Vigna, and, Zea.

5 One of skill will recognize that after the expression cassette is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

The particular sequences of SDFs identified are provided in the attached TABLE 1.

10 One of ordinary skill in the art, having this data, can obtain cloned DNA fragments, synthetic DNA fragments or polypeptides constituting desired sequences by recombinant methodology known in the art or described herein.

EXAMPLES

The invention is illustrated by way of the following examples. The invention is not
15 limited by these examples as the scope of the invention is defined solely by the claims following.

EXAMPLE 1: cDNA PREPARATION

A number of the nucleotide sequences disclosed in TABLE 1 herein as representative of the SDFs of the invention can be obtained by sequencing genomic DNA (gDNA) and/or cDNA
20 from corn plants grown from HYBRID SEED # 35A19, purchased from Pioneer Hi-Bred International, Inc., Supply Management, P.O. Box 256, Johnston, Iowa 50131-0256.

A number of the nucleotide sequences disclosed in TABLE 1 herein as representative of the SDFs of the invention can also be obtained by sequencing genomic DNA from
25 *Arabidopsis thaliana*, Wassilewskija ecotype or by sequencing cDNA obtained from mRNA from such plants as described below. This is a true breeding strain. Seeds of the plant are available from the Arabidopsis Biological Resource Center at the Ohio State University, under the accession number CS2360. Seeds of this plant were deposited under the terms and conditions of the Budapest Treaty at the American Type Culture Collection, Manassas, VA on August 31, 1999, and were assigned ATCC No. PTA-595.

30 Other methods for cloning full-length cDNA are described, for example, by Seki et al., *Plant Journal* 15:707-720 (1998) High-efficiency cloning of Arabidopsis full-length cDNA by biotinylated Cap trapper"; Maruyama et al., *Gene* 138:171 (1994) Oligo-capping a

simple method to replace the cap structure of eukaryotic mRNAs with oligoribonucleotides"; and WO 96/34981.

Tissues were, or each organ was, individually pulverized and frozen in liquid nitrogen. Next, the samples were homogenized in the presence of detergents and then centrifuged. The debris and nuclei were removed from the sample and more detergents were added to the sample. The sample was centrifuged and the debris was removed. Then the sample was applied to a 2M sucrose cushion to isolate polysomes. The RNA was isolated by treatment with detergents and proteinase K followed by ethanol precipitation and centrifugation. The polysomal RNA from the different tissues was pooled according to the following mass ratios: 15/15/1 for male inflorescences, female inflorescences and root, respectively. The pooled material was then used for cDNA synthesis by the methods described below.

Starting material for cDNA synthesis for the exemplary corn cDNA clones with sequences presented in TABLE 1 was poly(A)-containing polysomal mRNAs from inflorescences and root tissues of corn plants grown from HYBRID SEED # 35A19. Male inflorescences and female (pre-and post-fertilization) inflorescences were isolated at various stages of development. Selection for poly(A) containing polysomal RNA was done using oligo d(T) cellulose columns, as described by Cox and Goldberg, *Plant Molecular Biology: A Practical Approach*", pp. 1-35, Shaw ed., c. 1988 by IRL, Oxford. The quality and the integrity of the poly(A) RNAs were evaluated.

Starting material for cDNA synthesis for the exemplary *Arabidopsis* cDNA clones with sequences presented in TABLE 1 was polysomal RNA isolated from the top-most inflorescence tissues of *Arabidopsis thaliana* Wassilewskija (Ws.) and from roots of *Arabidopsis thaliana* Landsberg erecta (L. er.), also obtained from the Arabidopsis Biological Resource Center. Nine parts inflorescence to every part root was used, as measured by wet mass. Tissue was pulverized and exposed to liquid nitrogen. Next, the sample was homogenized in the presence of detergents and then centrifuged. The debris and nuclei were removed from the sample and more detergents were added to the sample. The sample was centrifuged and the debris was removed and the sample was applied to a 2M sucrose cushion to isolate polysomal RNA. Cox et al., *Plant Molecular Biology: A Practical Approach*", pp. 1-35, Shaw ed., c. 1988 by IRL, Oxford. The polysomal RNA was used for cDNA synthesis by the methods described below. Polysomal mRNA was then isolated as described above for corn cDNA. The quality of the RNA was assessed electrophoretically.

Following preparation of the mRNAs from various tissues as described above, selection of mRNA with intact 5' ends and specific attachment of an oligonucleotide tag to the 5' end of such mRNA was performed using either a chemical or enzymatic approach. Both techniques take advantage of the presence of the "cap" structure, which characterizes the 5' end of most
5 intact mRNAs and which comprises a guanosine generally methylated once, at the 7 position.

The chemical modification approach involves the optional elimination of the 2', 3'-cis diol of the 3' terminal ribose, the oxidation of the 2', 3'-cis diol of the ribose linked to the cap of the 5' ends of the mRNAs into a dialdehyde, and the coupling of the such obtained dialdehyde to a derivatized oligonucleotide tag. Further detail regarding the chemical approaches for
10 obtaining mRNAs having intact 5' ends are disclosed in International Application No. WO96/34981 published November 7, 1996.

The enzymatic approach for ligating the oligonucleotide tag to the intact 5' ends of mRNAs involves the removal of the phosphate groups present on the 5' ends of uncapped incomplete mRNAs, the subsequent decapping of mRNAs having intact 5' ends and the ligation
15 of the phosphate present at the 5' end of the decapped mRNA to an oligonucleotide tag. Further detail regarding the enzymatic approaches for obtaining mRNAs having intact 5' ends are disclosed in Dumas Milne Edwards J.B. (Doctoral Thesis of Paris VI University, Le clonage des ADNc complets: difficultés et perspectives nouvelles. Apports pour l'étude de la régulation de l'expression de la tryptophane hydroxylase de rat, 20 Dec. 1993), EP0 625572 and Kato *et al.*,
20 *Gene* 150:243-250 (1994).

In both the chemical and the enzymatic approach, the oligonucleotide tag has a restriction enzyme site (e.g. an EcoRI site) therein to facilitate later cloning procedures. Following attachment of the oligonucleotide tag to the mRNA, the integrity of the mRNA is examined by performing a Northern blot using a probe complementary to the oligonucleotide
25 tag.

For the mRNAs joined to oligonucleotide tags using either the chemical or the enzymatic method, first strand cDNA synthesis is performed using an oligo-dT primer with reverse transcriptase. This oligo-dT primer can contain an internal tag of at least 4 nucleotides, which can be different from one mRNA preparation to another. Methylated dCTP is used for cDNA
30 first strand synthesis to protect the internal EcoRI sites from digestion during subsequent steps. The first strand cDNA is precipitated using isopropanol after removal of RNA by alkaline hydrolysis to eliminate residual primers.

Second strand cDNA synthesis is conducted using a DNA polymerase, such as Klenow fragment and a primer corresponding to the 5' end of the ligated oligonucleotide. The primer is

typically 20-25 bases in length. Methylated dCTP is used for second strand synthesis in order to protect internal EcoRI sites in the cDNA from digestion during the cloning process.

Following second strand synthesis, the full-length cDNAs are cloned into a phagemid vector, such as pBlueScript™ (Stratagene). The ends of the full-length cDNAs are blunted with T4 DNA polymerase (Biolabs) and the cDNA is digested with EcoRI. Since methylated dCTP is used during cDNA synthesis, the EcoRI site present in the tag is the only hemi-methylated site; hence the only site susceptible to EcoRI digestion. In some instances, to facilitate subcloning, an Hind III adapter is added to the 3' end of full-length cDNAs.

The full-length cDNAs are then size fractionated using either exclusion chromatography (AcA, Biosepra) or electrophoretic separation which yields 3 to 6 different fractions. The full-length cDNAs are then directionally cloned either into pBlueScript™ using either the EcoRI and SmaI restriction sites or, when the Hind III adapter is present in the full-length cDNAs, the EcoRI and Hind III restriction sites. The ligation mixture is transformed, preferably by electroporation, into bacteria, which are then propagated under appropriate antibiotic selection.

Clones containing the oligonucleotide tag attached to full-length cDNAs are selected as follows.

The plasmid cDNA libraries made as described above are purified (e.g. by a column available from Qiagen). A positive selection of the tagged clones is performed as follows. Briefly, in this selection procedure, the plasmid DNA is converted to single stranded DNA using phage F1 gene II endonuclease in combination with an exonuclease (Chang et al., *Gene* 127:95 (1993)) such as exonuclease III or T7 gene 6 exonuclease. The resulting single stranded DNA is then purified using paramagnetic beads as described by Fry et al., *Biotechniques* 13: 124 (1992). Here the single stranded DNA is hybridized with a biotinylated oligonucleotide having a sequence corresponding to the 3' end of the oligonucleotide tag. Preferably, the primer has a length of 20-25 bases. Clones including a sequence complementary to the biotinylated oligonucleotide are selected by incubation with streptavidin coated magnetic beads followed by magnetic capture. After capture of the positive clones, the plasmid DNA is released from the magnetic beads and converted into double stranded DNA using a DNA polymerase such as ThermoSequenase™ (obtained from Amersham Pharmacia Biotech). Alternatively, protocols such as the Gene Trapper™ kit (Gibco BRL) can be used. The double stranded DNA is then transformed, preferably by electroporation, into bacteria. The percentage of positive clones having the 5' tag oligonucleotide is typically estimated to be between 90 and 98% from dot blot analysis.

Following transformation, the libraries are ordered in microtiter plates and sequenced. The *Arabidopsis* library was deposited at the American Type Culture Collection on January 7, 2000 as *E. coli* liba 010600" under the accession number **PTA-1161**.

EXAMPLE 2: SOUTHERN HYBRIDIZATIONS

The SDFs of the invention can be used in Southern hybridizations as described above. The following describes extraction of DNA from nuclei of plant cells, digestion of the nuclear DNA and separation by length, transfer of the separated fragments to membranes, preparation of probes for hybridization, hybridization and detection of the hybridized probe.

The procedures described herein can be used to isolate related polynucleotides or for diagnostic purposes. Moderate stringency hybridization conditions, as defined above, are described in the present example. These conditions result in detection of hybridization between sequences having at least 70% sequence identity. As described above, the hybridization and wash conditions can be changed to reflect the desired percentage of sequence identity between probe and target sequences that can be detected.

In the following procedure, a probe for hybridization is produced from two PCR reactions using two primers from genomic sequence of *Arabidopsis thaliana*. As described above, the particular template for generating the probe can be any desired template.

The first PCR product is assessed to validate the size of the primer to assure it is of the expected size. Then the product of the first PCR is used as a template, with the same pair of primers used in the first PCR, in a second PCR that produces a labeled product used as the probe.

Fragments detected by hybridization, or other bands of interest, can be isolated from gels used to separate genomic DNA fragments by known methods for further purification and/or characterization.

Buffers for nuclear DNA extraction

1. 10X HB

	1000 ml	
40 mM spermidine	10.2 g	Spermine (Sigma S-2876) and spermidine (Sigma S-2501)
10 mM spermine	3.5 g	Stabilize chromatin and the nuclear membrane

0.1 M EDTA (disodium)	37.2 g	EDTA inhibits nuclease
0.1 M Tris	12.1 g	Buffer
0.8 M KCl	59.6 g	Adjusts ionic strength for stability of nuclei

Adjust pH to 9.5 with 10 N NaOH. It appears that there is a nuclease present in leaves. Use of pH 9.5 appears to inactivate this nuclease.

2. 2 M sucrose (684 g per 1000 ml)

Heat about half the final volume of water to about 50°C. Add the sucrose slowly then bring the mixture to close to final volume; stir constantly until it has dissolved. Bring the solution to volume.

3. Sarkosyl solution (lyses nuclear membranes)

1000 ml

N-lauroyl sarcosine (Sarkosyl)

20.0 g

0.1 M Tris

12.1 g

0.04 M EDTA (Disodium)

14.9 g

Adjust the pH to 9.5 after all the components are dissolved and bring up to the proper volume.

4. 20% Triton X-100

80 ml Triton X-100

320 ml 1xHB (w/o β -ME and PMSF)

Prepare in advance; Triton takes some time to dissolve

A. Procedure

1. Prepare 1X H⁺ buffer (keep ice-cold during use)

	<u>1000 ml</u>
10X HB	100 ml
2 M sucrose	250 ml a non-ionic osmoticum
Water	634 ml

5 **Added just before use:**

100 mM PMSF*	10 ml a protease inhibitor; protects nuclear membrane proteins
β -mercaptoethanol	1 ml inactivates nuclease by reducing disulfide bonds

10

*100 mM PMSF

(phenyl methyl sulfonyl fluoride, Sigma P-7626)
(add 0.0875 g to 5 ml 100% ethanol)

15

2. Homogenize the tissue in a blender (use 300-400 ml of 1xHB per blender). Be sure that you use 5-10 ml of HB buffer per gram of tissue. Blenders generate heat so be sure to keep the homogenate cold. It is necessary to put the blenders in ice periodically.
3. Add the 20% Triton X-100 (25 ml per liter of homogenate) and gently stir on ice for 20 min. This lyses plastid, but not nuclear, membranes.
4. Filter the tissue suspension through several nylon filters into an ice-cold beaker. The first filtration is through a 250-micron membrane; the second is through an 85-micron membrane; the third is through a 50-micron membrane; and the fourth is through a 20-micron membrane. Use a large funnel to hold the filters. Filtration can be sped up by gently squeezing the liquid through the filters.
5. Centrifuge the filtrate at 1200 x g for 20 min. at 4°C to pellet the nuclei.

20

6. Discard the dark green supernatant. The pellet will have several layers to it. One is starch; it is white and gritty. The nuclei are gray and soft. In the early steps, there may be a dark green and somewhat viscous layer of chloroplasts.

Wash the pellets in about 25 ml cold H buffer (with Triton X-100) and resuspend by swirling gently and pipetting. After the pellets are resuspended.

Pellet the nuclei again at 1200 - 1300 x g. Discard the supernatant.

Repeat the wash 3-4 times until the supernatant has changed from a dark green to a pale green. This usually happens after 3 or 4 resuspensions. At this point, the pellet is typically grayish white and very slippery. The Triton X-100 in these repeated steps helps to destroy the chloroplasts and mitochondria that contaminate the prep.

Resuspend the nuclei for a final time in a total of 15 ml of H buffer and transfer the suspension to a sterile 125 ml Erlenmeyer flask.

7. Add 15 ml, dropwise, cold 2% Sarkosyl, 0.1 M Tris, 0.04 M EDTA solution (pH 9.5) while swirling gently. This lyses the nuclei. The solution will become very viscous.

8. Add 30 grams of CsCl and gently swirl at room temperature until the CsCl is in solution. The mixture will be gray, white and viscous.

9. Centrifuge the solution at 11,400 x g at 4°C for at least 30 min. The longer this spin is, the firmer the protein pellicle.

10. The result is typically a clear green supernatant over a white pellet, and (perhaps) under a protein pellicle. Carefully remove the solution under the protein pellicle and above the pellet. Determine the density of the solution by weighing 1 ml of solution and add CsCl if necessary to bring to 1.57 g/ml. The solution contains dissolved solids (sucrose etc) and the refractive index alone will not be an accurate guide to CsCl concentration.

11. Add 20 μ l of 10 mg/ml EtBr per ml of solution.
12. Centrifuge at 184,000 x g for 16 to 20 hours in a fixed-angle rotor.
13. Remove the dark red supernatant that is at the top of the tube with a plastic transfer pipette and discard. Carefully remove the DNA band with another transfer pipette.
5 The DNA band is usually visible in room light; otherwise, use a long wave UV light to locate the band.
14. Extract the ethidium bromide with isopropanol saturated with water and salt. Once the solution is clear, extract at least two more times to ensure that all of the EtBr is gone. Be very gentle, as it is very easy to shear the DNA at this step. This extraction may take a while because the DNA solution tends to be very viscous. If the solution is too viscous, dilute it with TE.
10
15. Dialyze the DNA for at least two days against several changes (at least three times) of TE (10 mM Tris, 1mM EDTA, pH 8) to remove the cesium chloride.
16. Remove the dialyzed DNA from the tubing. If the dialyzed DNA solution contains a lot of debris, centrifuge the DNA solution at least at 2500 x g for 10 min. and carefully transfer the clear supernatant to a new tube. Read the A260 concentration of the DNA.
15
17. Assess the quality of the DNA by agarose gel electrophoresis (1% agarose gel) of the DNA. Load 50 ng and 100 ng (based on the OD reading) and compare it with known and good quality DNA. Undigested lambda DNA and a lambda-HindIII-digested DNA are good molecular weight makers.
20

Protocol for Digestion of Genomic DNA

Protocol:

1. The relative amounts of DNA for different crop plants that provide approximately a balanced number of genome equivalent is given in Table 3. Note that due to the size

of the wheat genome, wheat DNA will be underrepresented. Lambda DNA provides a useful control for complete digestion.

2. Precipitate the DNA by adding 3 volumes of 100% ethanol. Incubate at -20°C for at least two hours. Yeast DNA can be purchased and made up at the necessary concentration, therefore no precipitation is necessary for yeast DNA.
3. Centrifuge the solution at $11,400 \times g$ for 20 min. Decant the ethanol carefully (be careful not to disturb the pellet). Be sure that the residual ethanol is completely removed either by vacuum desiccation or by carefully wiping the sides of the tubes with a clean tissue.
4. Resuspend the pellet in an appropriate volume of water. Be sure the pellet is fully resuspended before proceeding to the next step. This may take about 30 min.
5. Add the appropriate volume of 10X reaction buffer provided by the manufacturer of the restriction enzyme to the resuspended DNA followed by the appropriate volume of enzymes. Be sure to mix it properly by slowly swirling the tubes.
6. Set-up the lambda digestion-control for each DNA that you are digesting.
7. Incubate both the experimental and lambda digests overnight at 37°C . Spin down condensation in a microfuge before proceeding.
8. After digestion, add 2 μl of loading dye (typically 0.25% bromophenol blue, 0.25% xylene cyanol in 15% Ficoll or 30% glycerol) to the lambda-control digests and load in 1% TPE-agarose gel (TPE is 90 mM Tris-phosphate, 2 mM EDTA, pH 8). If the lambda DNA in the lambda control digests are completely digested, proceed with the precipitation of the genomic DNA in the digests.
9. Precipitate the digested DNA by adding 3 volumes of 100% ethanol and incubating in -20°C for at least 2 hours (preferably overnight).

EXCEPTION: *Arabidopsis* and yeast DNA are digested in an appropriate volume; they don't have to be precipitated.

10. Resuspend the DNA in an appropriate volume of TE (e.g., 22 μ l x 50 blots = 1100 μ l) and an appropriate volume of 10X loading dye (e.g., 2.4 μ l x 50 blots = 120 μ l). Be careful in pipetting the loading dye - it is viscous. Be sure you are pipetting the correct volume.

Table 3

Some guide points in digesting genomic DNA.

Species	Genome Size	Size Relative to <i>Arabidopsis</i>	Genome Equivalent to 2 μ g <i>Arabidopsis</i> DNA	Amount of DNA per blot
Arabidopsis	120 Mb	1X	1X	2 μ g
Brassica	1,100 Mb	9.2X	0.54X	10 μ g
Corn	2,800 Mb	23.3X	0.43X	20 μ g
Cotton	2,300 Mb	19.2X	0.52X	20 μ g
Oat	11,300 Mb	94X	0.11X	20 μ g
Rice	400 Mb	3.3X	0.75X	5 μ g
Soybean	1,100 Mb	9.2X	0.54X	10 μ g
Sugarbeet	758 Mb	6.3X	0.8X	10 μ g
Sweetclover	1,100 Mb	9.2X	0.54X	10 μ g
Wheat	16,000 Mb	133X	0.08X	20 μ g
Yeast	15 Mb	0.12X	1X	0.25 μ g

Protocol for Southern Blot Analysis

The digested DNA samples are electrophoresed in 1% agarose gels in 1x TPE buffer. Low voltage; overnight separations are preferred. The gels are stained with EtBr and photographed.

1. For blotting the gels, first incubate the gel in 0.25 N HCl (with gentle shaking) for about 15 min.
2. Then briefly rinse with water. The DNA is denatured by 2 incubations. Incubate (with shaking) in 0.5 M NaOH in 1.5 M NaCl for 15 min.
3. The gel is then briefly rinsed in water and neutralized by incubating twice (with shaking) in 1.5 M Tris pH 7.5 in 1.5 M NaCl for 15 min.
4. A nylon membrane is prepared by soaking it in water for at least 5 min, then in 6X SSC for at least 15 min. before use. (20x SSC is 175.3 g NaCl, 88.2 g sodium citrate per liter, adjusted to pH 7.0.)
5. The nylon membrane is placed on top of the gel and all bubbles in between are removed. The DNA is blotted from the gel to the membrane using an absorbent medium, such as paper toweling and 6x SCC buffer. After the transfer, the membrane may be lightly brushed with a gloved hand to remove any agarose sticking to the surface.
6. The DNA is then fixed to the membrane by UV crosslinking and baking at 80°C. The membrane is stored at 4°C until use.

B. Protocol for PCR Amplification of Genomic Fragments in Arabidopsis

Amplification procedures:

1. Mix the following in a 0.20 ml PCR tube or 96-well PCR plate:

Volume	Stock	Final Amount or Conc.
0.5 µl	~ 10 ng/µl genomic DNA ¹	5 ng
2.5 µl	10X PCR buffer	20 mM Tris, 50 mM KCl

¹ Arabidopsis DNA is used in the present experiment, but the procedure is a general one.

0.75 μ l	50 mM MgCl ₂	1.5 mM
1 μ l	10 pmol/ μ l Primer 1 (Forward)	10 pmol
1 μ l	10 pmol/ μ l Primer 2 (Reverse)	10 pmol
0.5 μ l	5 mM dNTPs	0.1 mM
0.1 μ l	5 units/ μ l Platinum Taq™ (Life Technologies, Gaithersburg, MD) DNA Polymerase	1 units
(to 25 μ l)	Water	

2. The template DNA is amplified using a Perkin Elmer 9700 PCR machine:

1) 94°C for 10 min. followed by

2)	3)	4)
5 cycles:	5 cycles:	25 cycles:
94 °C - 30 sec	94 °C - 30 sec	94 °C - 30 sec
62 °C - 30 sec	58 °C - 30 sec	53 °C - 30 sec
72 °C - 3 min	72 °C - 3 min	72 °C - 3 min

5) 72°C for 7 min. Then the reactions are stopped by chilling to 4°C.

The procedure can be adapted to a multi-well format if necessary.

5 Quantification and Dilution of PCR Products:

1. The product of the PCR is analyzed by electrophoresis in a 1% agarose gel. A linearized plasmid DNA can be used as a quantification standard (usually at 50, 100,

200, and 400 ng). These will be used as references to approximate the amount of PCR products. HindIII-digested Lambda DNA is useful as a molecular weight marker. The gel can be run fairly quickly; e.g., at 100 volts. The standard gel is examined to determine that the size of the PCR products is consistent with the expected size and if there are significant extra bands or smeary products in the PCR reactions.

2. The amounts of PCR products can be estimated on the basis of the plasmid standard.
3. For the small number of reactions that produce extraneous bands, a small amount of DNA from bands with the correct size can be isolated by dipping a sterile 10- μ l tip into the band while viewing through a UV Transilluminator. The small amount of agarose gel (with the DNA fragment) is used in the labeling reaction.

C. Protocol for PCR-DIG-Labeling of DNA

Solutions:

Reagents in PCR reactions (diluted PCR products, 10X PCR Buffer, 50 mM $MgCl_2$, 5 U/ μ l Platinum Taq Polymerase, and the primers)

10X dNTP + DIG-11-dUTP [1:5]: (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 1.65 mM dTTP, 0.35 mM DIG-11-dUTP)

10X dNTP + DIG-11-dUTP [1:10]: (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 1.81 mM dTTP, 0.19 mM DIG-11-dUTP)

10X dNTP + DIG-11-dUTP [1:15]: (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 1.875 mM dTTP, 0.125 mM DIG-11-dUTP)

TE buffer (10 mM Tris, 1 mM EDTA, pH 8)

Maleate buffer: In 700 ml of deionized distilled water, dissolve 11.61 g maleic acid and 8.77 g NaCl. Add NaOH to adjust the pH to 7.5. Bring the volume to 1 L. Stir for 15 min. and sterilize.

10% blocking solution: In 80 ml deionized distilled water, dissolve 1.16g maleic acid. Next, add NaOH to adjust the pH to 7.5. Add 10 g of the blocking reagent powder (Boehringer Mannheim, Indianapolis, IN, Cat. no. 1096176). Heat to 60°C while stirring to dissolve the powder. Adjust the volume to 100 ml with water. Stir and sterilize.

5

1% blocking solution: Dilute the 10% stock to 1% using the maleate buffer.

Buffer 3 (100 mM Tris, 100 mM NaCl, 50 mM MgCl₂, pH9.5). Prepared from autoclaved solutions of 1M Tris pH 9.5, 5 M NaCl, and 1 M MgCl₂ in autoclaved distilled water.

Procedure:

1. PCR reactions are performed in 25 μ l volumes containing:

PCR buffer	1X
MgCl ₂	1.5 mM
10X dNTP + DIG-11-dUTP	1X (please see the note below)
Platinum Taq™ Polymerase	1 unit
10 pg probe DNA	
10 pmol primer 1	

Note:Use for:

10X dNTP + DIG-11-dUTP (1:5)	< 1 kb
10X dNTP + DIG-11-dUTP (1:10)	1 kb to 1.8 kb
10X dNTP + DIG-11-dUTP (1:15)	> 1.8 kb

2. The PCR reaction uses the following amplification cycles:

- 1) 94°C for 10 min.

2) 5 cycles:	3) 5 cycles:	4) 25 cycles:
95°C - 30 sec	95°C - 30 sec	95°C - 30 sec
61°C - 1 min	59°C - 1 min	51°C - 1 min
73°C - 5 min	75°C - 5 min	73°C - 5 min

- 5) 72°C for 8 min. The reactions are terminated by chilling to 4°C (hold).

3. The products are analyzed by electrophoresis- in a 1% agarose gel, comparing to an aliquot of the unlabelled probe starting material.
4. The amount of DIG-labeled probe is determined as follows:

Make serial dilutions of the diluted control DNA in dilution buffer (TE: 10 mM Tris and 1 mM EDTA, pH 8) as shown in the following table:

DIG-labeled control DNA starting conc.	Stepwise Dilution	Final Conc. (Dilution Name)
5 ng/ μ l	1 μ l in 49 μ l TE	100 pg/ μ l (A)
100 pg/ μ l (A)	25 μ l in 25 μ l TE	50 pg/ μ l (B)
50 pg/ μ l (B)	25 μ l in 25 μ l TE	25 pg/ μ l (C)
25 pg/ μ l (C)	20 μ l in 30 μ l TE	10 pg/ μ l (D)

- a. Serial deletions of a DIG-labeled standard DNA ranging from 100 pg to 10 pg are spotted onto a positively charged nylon membrane, marking the membrane lightly with a pencil to identify each dilution.
- b. Serial dilutions (e.g., 1:50, 1:2500, 1:10,000) of the newly labeled DNA probe are spotted.
- c. The membrane is fixed by UV crosslinking.
- d. The membrane is wetted with a small amount of maleate buffer and then incubated in 1% blocking solution for 15 min at room temp.
- e. The labeled DNA is then detected using alkaline phosphatase conjugated anti-DIG antibody (Boehringer Mannheim, Indianapolis, IN, cat. no. 1093274) and an NBT substrate according to the manufacture's instruction.
- f. Spot intensities of the control and experimental dilutions are then compared to estimate the concentration of the PCR-DIG-labeled probe.

D. Prehybridization and Hybridization of Southern BlotsSolutions:

100% Formamide

purchased from Gibco

20X SSC

(1X = 0.15 M NaCl, 0.015 M Na₃citrate)

per L:

175 g NaCl

87.5 g Na₃citrate·2H₂O

20% Sarkosyl (N-lauroyl-sarcosine)

20% SDS (sodium dodecyl sulphate)

10% Blocking Reagent: In 80 ml deionized distilled water, dissolve 1.16 g maleic acid. Next, add NaOH to adjust the pH to 7.5. Add 10 g of the blocking reagent powder. Heat to 60°C while stirring to dissolve the powder. Adjust the volume to 100 ml with water. Stir and sterilize.

Prehybridization Mix:

Final Concentration	Components	Volume (per 100 ml)	Stock
50%	Formamide	50 ml	100%
5X	SSC	25 ml	20X
0.1%	Sarkosyl	0.5 ml	20%
0.02%	SDS	0.1 ml	20%
2%	Blocking Reagent	20 ml	10%
	Water	4.4 ml	

General Procedures:

- Place the blot in a heat-sealable plastic bag and add an appropriate volume of prehybridization solution (30 ml/100cm²) at room temperature. Seal the bag with a heat sealer, avoiding bubbles as much as possible. Lay down the bags in a large plastic tray (one tray can accommodate at least 4-5 bags). Ensure that the bags are

lying flat in the tray so that the prehybridization solution is evenly distributed throughout the bag. Incubate the blot for at least 2 hours with gentle agitation using a waver shaker.

2. Denature DIG-labeled DNA probe by incubating for 10 min. at 98°C using the PCR machine and immediately cool it to 4°C.

3. Add probe to prehybridization solution (25 ng/ml; 30 ml = 750 ng total probe) and mix well but avoid foaming. Bubbles may lead to background.

4. Pour off the prehybridization solution from the hybridization bags and add new prehybridization and probe solution mixture to the bags containing the membrane.

5. Incubate with gentle agitation for at least 16 hours.

6. Proceed to medium stringency post-hybridization wash:

Three times for 20 min. each with gentle agitation using 1X SSC, 1% SDS at 60°C.

All wash solutions must be prewarmed to 60°C. Use about 100 ml of wash solution per membrane.

7. To avoid background keep the membranes fully submerged to avoid drying in spots; agitate sufficiently to avoid having membranes stick to one another.

7. After the wash, proceed to immunological detection and CSPD development.

E. Procedure for Immunological Detection with CSPD

Solutions:

Buffer 1: Maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl; adjusted to pH 7.5 with NaOH)

Washing buffer: Maleic acid buffer with 0.3% (v/v) Tween 20.

Blocking stock solution

10% blocking reagent in buffer 1. Dissolve (10X concentration): blocking reagent powder (Boehringer Mannheim, Indianapolis, IN, cat. no. 1096176) by constantly stirring on a 65°C heating block or heat in a microwave, autoclave and store at 4°C.

Buffer 2

(1X blocking solution):

Dilute the stock solution 1:10 in Buffer 1.

Detection buffer:

0.1 M Tris, 0.1 M NaCl, pH 9.5

Procedure:

1. After the post-hybridization wash the blots are briefly rinsed (1-5 min.) in the maleate washing buffer with gentle shaking.
2. Then the membranes are incubated for 30 min. in Buffer 2 with gentle shaking.
3. Anti-DIG-AP conjugate (Boehringer Mannheim, Indianapolis, IN, cat. no. 1093274) at 75 mU/ml (1:10,000) in Buffer 2 is used for detection. 75 ml of solution can be used for 3 blots.
4. The membrane is incubated for 30 min. in the antibody solution with gentle shaking.
5. The membrane are washed twice in washing buffer with gentle shaking. About 250 ml is used per wash for 3 blots.
6. The blots are equilibrated for 2-5 min in 60 ml detection buffer.
7. Dilute CSPD (1:200) in detection buffer. (This can be prepared ahead of time and stored in the dark at 4°C).

The following steps must be done individually. Bags (one for detection and one for exposure) are generally cut and ready before doing the following steps.

8. The blot is carefully removed from the detection buffer and excess liquid removed without drying the membrane. The blot is immediately placed in a bag and 1.5 ml of CSPD solution is added. The CSPD solution can be spread over the membrane. Bubbles present at the edge and on the surface of the blot are typically removed by gentle rubbing. The membrane is incubated for 5 min. in CSPD solution.
9. Excess liquid is removed and the membrane is blotted briefly (DNA side up) on Whatman 3MM paper. Do not let the membrane dry completely.
10. Seal the damp membrane in a hybridization bag and incubate for 10 min at 37°C to enhance the luminescent reaction.
11. Expose for 2 hours at room temperature to X-ray film. Multiple exposures can be taken. Luminescence continues for at least 24 hours and signal intensity increases during the first hours.

Example 3: Transformation of Carrot Cells

Transformation of plant cells can be accomplished by a number of methods, as described above. Similarly, a number of plant genera can be regenerated from tissue culture following transformation. Transformation and regeneration of carrot cells as described herein is illustrative.

Single cell suspension cultures of carrot (*Daucus carota*) cells are established from hypocotyls of cultivar Early Nantes in B₅ growth medium (O.L. Gamborg et al., *Plant Physiol.* 45:372 (1970)) plus 2,4-D and 15 mM CaCl₂ (B₅-44 medium) by methods known in the art. The suspension cultures are subcultured by adding 10 ml of the suspension culture to 40 ml of B₅-44 medium in 250 ml flasks every 7 days and are maintained in a shaker at 150 rpm at 27 °C in the dark.

The suspension culture cells are transformed with exogenous DNA as described by Z. Chen et al. *Plant Mol. Bio.* 36:163 (1998). Briefly, 4-days post-subculture cells are incubated with cell wall digestion solution containing 0.4 M sorbitol, 2% driselase, 5mM MES (2-[N-Morpholino] ethanesulfonic acid) pH 5.0 for 5 hours. The digested cells are pelleted gently at 60 xg for 5 min. and washed twice in W5 solution containing 154 mM NaCl, 5 mM KCl, 125 mM CaCl₂ and 5mM glucose, pH 6.0. The protoplasts are suspended in MC solution

containing 5 mM MES, 20 mM CaCl₂, 0.5 M mannitol, pH 5.7 and the protoplast density is adjusted to about 4×10^6 protoplasts per ml.

15-60 µg of plasmid DNA is mixed with 0.9 ml of protoplasts. The resulting suspension is mixed with 40% polyethylene glycol (MW 8000, PEG 8000), by gentle inversion a few times at room temperature for 5 to 25 min. Protoplast culture medium known in the art is added into the PEG-DNA-protoplast mixture. Protoplasts are incubated in the culture medium for 24 hour to 5 days and cell extracts can be used for assay of transient expression of the introduced gene. Alternatively, transformed cells can be used to produce transgenic callus, which in turn can be used to produce transgenic plants, by methods known in the art. See, for example, Nomura and Komamine, *Plt. Phys.* 79:988-991 (1985), *Identification and Isolation of Single Cells that Produce Somatic Embryos in Carrot Suspension Cultures*.

An additional deposit, PTA-1411, of an *E. coli* Library, *E. coli*LibA021800, was made at the American Type Culture Collection in Manassas, Virginia, USA on February 22, 2000 to meet the requirements of Budapest Treaty for the international recognition of the deposit of microorganisms. This deposit was assigned ATCC accession no. PTA-1411.

The invention being thus described, it will be apparent to one of ordinary skill in the art that various modifications of the materials and methods for practicing the invention can be made. Such modifications are to be considered within the scope of the invention as defined by the following claims.

Each of the references from the patent and periodical literature cited herein is hereby expressly incorporated in its entirety by such citation.

Table 1

>5541651

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	len =	37560	nex =	61		
5	Term	351	143	-	0	
	Intr	795	738	-	0	
	Intr	971	922	-	0	
	Intr	1292	1086	-	0	
10	Intr	2075	1655	-	0	
	Intr	2936	2841	-	0	
	Init	3468	3148	-	0	
	Term	8256	7580	-	1	
15	Intr	8495	8342	-	1	
	Intr	10104	8596	-	1	
	Intr	10243	10200	-	1	
	Init	10453	10327	-	1	
20	Sngl	15598	15326	-	2	
	Sngl	17736	16462	-	3	
	Term	24957	24846	-	4	
25	Intr	25196	25114	-	4	
	Intr	25380	25300	-	4	
	Intr	25525	25469	-	4	
	Intr	25747	25691	-	4	
	Intr	25939	25843	-	4	
30	Intr	26088	26018	-	4	
	Intr	26302	26174	-	4	
	Intr	26598	26398	-	4	
	Intr	26854	26702	-	4	
	Intr	27206	27036	-	4	
35	Intr	27351	27289	-	4	
	Intr	27701	27590	-	4	
	Intr	27930	27791	-	4	
	Intr	28293	28081	-	4	
	Intr	28517	28419	-	4	
40	Intr	28759	28640	-	4	
	Intr	29124	28919	-	4	
	Intr	29419	29242	-	4	
	Intr	29567	29507	-	4	
	Intr	29754	29645	-	4	
45	Intr	29980	29849	-	4	
	Intr	30235	30068	-	4	
	Intr	30512	30333	-	4	
	Intr	30670	30633	-	4	
	Intr	31006	30905	-	4	
50	Intr	31612	31501	-	4	
	Intr	31870	31736	-	4	
	Intr	32110	31974	-	4	
	Intr	32355	32206	-	4	
	Intr	32640	32481	-	4	
55	Intr	32775	32717	-	4	
	Intr	33232	33076	-	4	
	Intr	33480	33335	-	4	
	Intr	33698	33555	-	4	
	Intr	33927	33799	-	4	
60	Intr	34294	34256	-	4	

846

	Intr	34557	34501	-	4
	Intr	35392	35340	-	4
	Intr	35592	35466	-	4
	Intr	35779	35679	-	4
5	Intr	36117	35991	-	4
	Intr	36609	36499	-	4
	Intr	36851	36701	-	4
	Intr	37011	36959	-	4
	Intr	37176	37102	-	4
10	Init	37390	37313	-	4
<p>>5541653 /</p>					
15	len =	79320	nex =	127	
	Sngl	203	215	+	0
	Init	2597	3003	+	1
	Term	3394	3721	+	1
20	Sngl	4128	4322	+	2
	Init	4924	5133	+	3
	Intr	5240	5302	+	3
25	Intr	5391	5672	+	3
	Intr	5800	5865	+	3
	Intr	5974	6102	+	3
	Intr	6231	6524	+	3
	Intr	6765	6827	+	3
30	Intr	6972	7049	+	3
	Intr	7141	7209	+	3
	Intr	7309	7392	+	3
	Intr	7531	7587	+	3
	Intr	7671	7747	+	3
35	Intr	7949	8030	+	3
	Intr	8121	8237	+	3
	Intr	8550	8683	+	3
	Intr	8852	8921	+	3
	Term	9024	9092	+	3
40	Sngl	11309	9474	-	4
	Init	11464	11808	+	5
	Intr	12005	12139	+	5
45	Intr	12237	12428	+	5
	Intr	12541	12602	+	5
	Intr	12725	12885	+	5
	Intr	12967	13115	+	5
	Intr	13194	13334	+	5
50	Intr	13416	13568	+	5
	Intr	14271	16052	+	5
	Intr	16442	16503	+	5
	Intr	16783	16967	+	5
	Intr	17266	17648	+	5
55	Intr	17732	18016	+	5
	Intr	18112	18347	+	5
	Term	18432	18609	+	5
60	Init	21616	21783	-	6
	Intr	21972	22366	+	6
	Intr	22453	22537	+	6

	Intr	22634	22856	+	6
	Intr	22944	23047	+	6
	Intr	23131	23221	+	6
	Intr	23328	23482	+	6
5	Intr	23656	24657	+	6
	Intr	24741	25127	+	6
	Term	25212	25508	+	6
	Term	26400	26248	-	7
10	Intr	26591	26485	-	7
	Intr	26878	26674	-	7
	Intr	27285	26952	-	7
	Intr	27556	27372	-	7
	Intr	27832	27637	-	7
15	Init	28924	27912	-	7
	Term	32372	32284	-	8
	Intr	32622	32480	-	8
	Intr	32841	32719	-	8
20	Intr	33040	32941	-	8
	Intr	33217	33135	-	8
	Intr	33471	33319	-	8
	Init	33983	33634	-	8
25	Sngl	35765	35103	-	9
	Term	36432	35963	-	10
	Intr	36698	36497	-	10
	Intr	37001	36902	-	10
30	Intr	37282	37196	-	10
	Intr	37647	37406	-	10
	Intr	37914	37832	-	10
	Intr	38349	38232	-	10
	Intr	38964	38576	-	10
35	Init	40843	39145	-	10
	Init	43542	43562	+	11
	Intr	43667	43867	+	11
	Intr	44142	44291	+	11
40	Intr	44386	44571	+	11
	Intr	44663	44743	+	11
	Intr	44910	45110	+	11
	Intr	45226	45366	+	11
	Term	45456	45653	+	11
45	Init	46353	46741	+	12
	Intr	46913	47010	+	12
	Intr	47095	47160	+	12
	Term	47519	48153	+	12
50	Sngl	48876	50090	+	13
	Init	52171	52413	+	14
	Intr	52540	52713	+	14
55	Intr	52865	53033	+	14
	Intr	53180	53241	+	14
	Intr	53331	53452	+	14
	Intr	53623	53740	+	14
	Term	53836	53973	+	14
60	Sngl	58340	59602	+	15

	Term	60245	60133	-	16
	Intr	60439	60334	-	16
	Intr	61880	61519	-	16
5	Init	62208	61986	-	16
	Term	63156	62911	-	17
	Intr	63595	63254	-	17
	Intr	63776	63660	-	17
10	Intr	64014	63850	-	17
	Init	64788	64612	-	17
	Init	65271	65491	+	18
	Term	65625	66057	+	18
15	Term	66668	66504	-	19
	Intr	66933	66778	-	19
	Intr	67142	67044	-	19
	Intr	67570	67248	-	19
20	Init	68343	67659	-	19
	Sngl	69061	68911	-	20
	Init	70351	70437	+	21
	Intr	70537	70606	+	21
	Intr	70706	70784	+	21
	Intr	71159	71240	+	21
	Intr	71441	71549	+	21
	Intr	71688	71776	+	21
	Intr	71853	71917	+	21
	Intr	72034	72112	+	21
	Term	72192	72359	+	21
	Init	72879	73109	+	22
	Intr	73419	73646	+	22
	Intr	73748	73821	+	22
	Intr	73963	74122	+	22
	Intr	74217	74336	+	22
	Intr	74453	74561	+	22
	Intr	74724	74879	+	22
	Term	74965	75032	+	22
	Sngl	78028	79311	+	23
45	>5541654	/			
	len =	58920	nex =	63	
	Init	58	244	+	3
	Term	3227	3330	+	0
	Init	5408	5527	+	1
	Intr	5889	5940	+	1
	Intr	6067	6147	+	1
	Intr	6238	6356	-	1
	Intr	6668	6887	+	1
	Intr	6973	7091	+	1
	Term	7285	8040	+	1
	Sngl	8735	9310	+	2

	Term	9809	9640	-	3
	Init	10251	10188	-	3
	Sngl	10639	10821	+	4
5	Term	12075	11900	-	5
	Init	12465	12402	-	5
	Sngl	13513	14094	+	6
10	Init	15064	15604	+	7
	Intr	16038	16108	+	7
	Intr	16260	18224	+	7
	Term	19124	19150	+	7
15	Init	21109	21501	+	8
	Intr	21566	22902	+	8
	Intr	22974	23084	+	8
	Intr	23177	23453	+	8
20	Intr	23543	23631	+	8
	Intr	23713	23821	+	8
	Intr	23906	24130	+	8
	Intr	24199	24324	+	8
	Intr	24399	24497	+	8
25	Term	24573	24764	+	8
	Term	24836	25447	+	8
	Init	28588	29266	+	9
	Intr	30133	30823	+	9
30	Intr	30902	31263	+	9
	Intr	31288	31551	+	9
	Intr	31584	33236	+	9
	Intr	33292	33923	+	9
	Intr	34136	34828	+	9
35	Term	35080	35628	+	9
	Term	37540	37491	-	10
	Intr	37732	37643	-	10
	Init	38328	38238	-	10
40	Sngl	39544	39741	+	11
	Term	41267	40966	-	12
	Intr	41929	41738	-	12
45	Intr	42087	42001	-	12
	Intr	42553	42295	-	12
	Init	43798	43166	-	12
	Sngl	45500	45108	-	13
50	Sngl	46583	46020	-	14
	Term	48039	47391	-	15
	Init	48546	48257	-	15
55	Term	52521	52104	-	16
	Intr	53417	53288	-	16
	Init	53665	53527	-	16
60	Term	56895	56818	-	17
	Intr	57100	56966	-	17

850

	Intr	57356	57204	-	17
	Intr	57673	57475	-	17
	Intr	57938	57901	-	17
	Intr	58231	58060	-	17
5	Intr	58512	58390	-	17
	Init	58838	58602	-	17

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10 len = 36840 nex = 69

	Init	1223	1228	+	0
	Intr	1302	1380	+	0
	Term	1441	1901	+	0

15

	Init	2569	2832	+	1
	Intr	2932	3498	+	1
	Intr	3578	3757	-	1

20

	Intr	3917	4020	+	1
	Intr	4094	4205	+	1
	Intr	4285	4383	+	1
	Intr	4471	4590	+	1
	Term	4684	4800	+	1

25

	Term	7356	7204	-	2
	Intr	7705	7439	-	2
	Intr	7993	7787	-	2

30

	Intr	8251	8093	-	2
	Intr	8487	8350	-	2
	Intr	8679	8574	-	2
	Intr	8932	8765	-	2

35

	Intr	9244	9054	-	2
	Intr	9660	9436	-	2
	Intr	9933	9816	-	2

40

	Intr	10187	10024	-	2
	Intr	10381	10271	-	2
	Intr	10589	10484	-	2
	Intr	10872	10767	-	2

45

	Intr	11196	11026	-	2
	Intr	11402	11281	-	2
	Intr	11635	11501	-	2
	Intr	11799	11719	-	2
	Intr	12092	11881	-	2

50

	Intr	12645	12156	-	2
	Intr	13346	13240	-	2
	Init	16970	14193	-	2

55

	Init	18232	18596	+	3
	Intr	18799	19018	+	3
	Intr	19099	19423	+	3
	Intr	19512	19906	+	3
	Intr	20122	20402	+	3

60

	Intr	20471	20502	+	3
	Intr	20852	20983	+	3
	Intr	21370	21420	+	3
	Term	21557	21609	+	3

	Init	23028	23370	+	4
	Intr	23451	24565	+	4
	Term	24656	24730	+	4

851

	Sngl	25809	26408	+	5
	Init	27959	29286	+	6
5	Intr	29376	29542	+	6
	Intr	29628	29866	+	6
	Term	29960	30574	+	6
	Term	31091	30909	-	7
10	Intr	31376	31248	-	7
	Intr	31936	31464	-	7
	Intr	32209	32032	-	7
	Intr	32622	32402	-	7
	Init	32755	32662	-	7
15	Term	33582	33472	-	8
	Intr	33809	33802	-	8
	Intr	34127	34028	-	8
	Intr	34256	34225	-	8
20	Intr	34588	34371	-	8
	Intr	34782	34667	-	8
	Intr	35122	34869	-	8
	Intr	35540	35465	-	8
	Intr	35690	35629	-	8
25	Intr	35859	35799	-	8
	Intr	35955	35921	-	8
	Init	36126	36033	-	8
	Sngl	36839	36687	-	9
30	>5541656	/			
	len =	71580	nex =	90	
35	Sngl	137	140	+	0
	Init	176	370	+	1
	Intr	467	736	+	1
	Term	992	1930	+	1
40	Init	4090	4284	+	2
	Intr	4333	4377	+	2
	Intr	4437	4706	+	2
	Term	4957	5904	+	2
45	Init	9007	9363	+	3
	Term	9243	9296	+	3
	Init	9716	9916	+	4
50	Intr	10072	10512	+	4
	Intr	10613	10684	+	4
	Term	10983	11930	+	4
	Init	13084	13278	+	5
55	Intr	13375	13818	+	5
	Intr	13908	14279	+	5
	Intr	14361	14583	+	5
	Intr	14996	15084	+	5
	Intr	15443	15505	+	5
60	Intr	15833	15847	+	5
	Intr	17101	17331	+	5
	Intr	17429	17872	+	5

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	Term	17994	18245	+	5
	Init	19401	19601	+	6
	Intr	19703	20140	+	6
5	Term	20279	20530	+	6
	Sngl	21514	21699	+	7
	Init	23658	23852	+	8
10	Intr	24183	24638	+	8
	Term	24861	25112	+	8
	Init	26164	26358	+	9
	Intr	26543	26998	+	9
15	Term	27112	27363	+	9
	Init	30600	30746	+	10
	Intr	30850	31656	+	10
	Intr	31754	32206	+	10
20	Intr	32289	32750	+	10
	Term	32845	33093	+	10
	Term	33915	33667	-	11
	Intr	34455	34006	-	11
25	Intr	35002	34541	-	11
	Init	35280	35086	-	11
	Sngl	37170	37322	+	12
	Init	37477	37515	+	13
	Intr	37805	38038	+	13
	Intr	38121	38417	+	13
	Intr	38504	38671	+	13
	Intr	38783	38950	+	13
35	Intr	39040	39117	+	13
	Intr	39191	39257	+	13
	Intr	39379	39476	+	13
	Intr	39566	39635	+	13
	Intr	39725	39795	+	13
40	Intr	39881	39930	+	13
	Intr	40024	40136	+	13
	Term	40241	40287	+	13
	Term	42156	41533	-	14
45	Intr	42426	42244	-	14
	Init	43060	42698	-	14
	Term	50406	50372	-	15
	Intr	50550	50483	-	15
50	Intr	50902	50770	-	15
	Intr	51275	50995	-	15
	Init	51779	51487	-	15
	Term	55279	54721	-	16
55	Init	55765	55395	-	16
	Term	56422	56418	-	17
	Intr	56753	56657	-	17
	Intr	57200	57037	-	17
60	Init	58404	57292	-	17

853

	Sngl	62987	62157	-	18
	Term	64148	63861	-	19
	Intr	64373	64323	-	19
5	Intr	64724	64596	-	19
	Intr	64936	64809	-	19
	Intr	65273	65014	-	19
	Intr	65562	65388	-	19
	Intr	65948	65706	-	19
10	Intr	66261	66020	-	19
	Intr	67497	67403	-	19
	Intr	67685	67575	-	19
	Intr	67832	67782	-	19
	Intr	68202	68056	-	19
15	Intr	68436	68311	-	19
	Intr	68778	68538	-	19
	Intr	69068	68893	-	19
	Intr	69642	69388	-	19
	Init	69988	69724	-	19
20	Sngl	70997	71323	+	20
	>5541657		/		
25	len =	20760	nex =	51	
	Init	889	991	+	0
	Intr	1069	1164	+	0
	Intr	1247	1302	+	0
30	Intr	1398	1521	+	0
	Intr	1611	1673	+	0
	Intr	1774	1833	+	0
	Intr	1933	2094	+	0
	Intr	2173	2246	+	0
35	Intr	2324	2417	+	0
	Term	2500	2640	+	0
	Init	5379	5521	+	1
	Intr	5591	5690	+	1
40	Intr	5772	5867	+	1
	Intr	5948	6003	+	1
	Intr	6096	6219	+	1
	Intr	6272	6334	+	1
	Intr	6376	6434	+	1
45	Intr	6563	6749	+	1
	Intr	6818	6891	+	1
	Intr	6965	7058	+	1
	Intr	7135	7271	+	1
	Term	7490	7496	+	1
50	Term	8030	7941	-	2
	Intr	8384	8298	-	2
	Intr	8568	8460	-	2
	Intr	9171	9100	-	2
55	Init	9510	9239	-	2
	Init	13166	13342	+	3
	Intr	13759	13818	+	3
	Intr	14103	14426	+	3
60	Intr	14519	14620	+	3
	Intr	14691	14750	+	3

	Intr	14842	15071	+	3
	Intr	15151	15274	+	3
	Intr	15365	15545	+	3
	Intr	15752	15773	+	3
5	Intr	15865	15900	+	3
	Intr	15974	16076	+	3
	Intr	16177	16272	+	3
	Intr	16373	16419	+	3
10	Intr	16490	16613	+	3
	Intr	16708	16770	+	3
	Intr	16865	17167	+	3
	Intr	17248	17321	+	3
	Intr	17407	17500	+	3
	Intr	17593	17729	+	3
15	Intr	17803	17883	+	3
	Intr	17968	18073	+	3
	Term	18174	18254	+	3
	Term	20027	19932	-	4
20	Init	20581	20524	-	4
	>5541658 /				
	len =	63000	nex =	120	
25	Init	1	131	+	0
	Term	167	229	+	0
	Term	1101	877	-	1
30	Intr	1361	1182	-	1
	Intr	1619	1503	-	1
	Intr	1833	1768	-	1
	Intr	2009	1918	-	1
	Intr	2250	2187	-	1
35	Intr	2430	2352	-	1
	Intr	2648	2509	-	1
	Intr	2804	2723	-	1
	Intr	3030	2927	-	1
	Intr	3270	3193	-	1
40	Intr	3516	3423	-	1
	Intr	3700	3603	-	1
	Init	4469	3912	-	1
	Term	5494	5385	-	2
45	Intr	5886	5583	-	2
	Intr	6288	5983	-	2
	Intr	6904	6395	-	2
	Intr	7184	7020	-	2
	Intr	7979	7272	-	2
50	Intr	8105	8019	-	2
	Intr	10111	8202	-	2
	Init	10616	10586	-	2
	Term	12494	12379	-	3
55	Intr	12819	12580	-	3
	Intr	12967	12904	-	3
	Intr	13621	13050	-	3
	Intr	13960	13666	-	3
	Intr	14239	14075	-	3
60	Intr	14962	14348	-	3
	Intr	15127	15041	-	3

	Init	17081	15240	-	3
	Term	18355	18225	-	4
	Intr	18679	18440	-	4
5	Intr	18828	18765	-	4
	Intr	19820	18912	-	4
	Intr	20166	20002	-	4
	Intr	20879	20268	-	4
	Intr	21046	20960	-	4
10	Intr	22578	21173	-	4
	Intr	22969	22699	-	4
	Intr	23073	23009	-	4
	Intr	24013	23926	-	4
	Init	25480	24314	-	4
15	Term	29987	29676	-	5
	Intr	30515	30477	-	5
	Init	30892	30668	-	5
20	Init	32168	32195	+	6
	Term	32330	32979	+	6
	Term	36474	36020	-	7
	Intr	36633	36558	-	7
25	Intr	36805	36723	-	7
	Init	38996	37385	-	7
	Sngl	40544	39360	-	8
30	Term	41055	40926	-	9
	Intr	41208	41145	-	9
	Intr	41425	41303	-	9
	Intr	41700	41572	-	9
	Intr	41804	41773	-	9
35	Intr	42090	42043	-	9
	Intr	42273	42237	-	9
	Intr	42418	42349	-	9
	Intr	42523	42507	-	9
	Intr	42699	42626	-	9
40	Intr	42947	42846	-	9
	Intr	43102	43029	-	9
	Intr	43374	43275	-	9
	Init	43558	43504	-	9
45	Term	44946	44626	-	10
	Intr	45823	45707	-	10
	Intr	45992	45896	-	10
	Intr	46187	46102	-	10
	Intr	46366	46295	-	10
50	Intr	46567	46419	-	10
	Intr	46806	46699	-	10
	Intr	47018	46958	-	10
	Intr	47181	47122	-	10
	Intr	47370	47310	-	10
55	Intr	47548	47457	-	10
	Intr	47866	47780	-	10
	Intr	48045	47962	-	10
	Intr	48314	48249	-	10
	Intr	48539	48426	-	10
60	Intr	48821	48667	-	10
	Intr	49102	49040	-	10

856

	Init	49332	49218	-	10
	Init	50117	50206	+	11
	Term	50310	51170	+	11
5	Init	51605	51735	+	12
	Intr	52114	52220	+	12
	Intr	52289	52330	+	12
	Intr	52425	52512	+	12
10	Intr	52786	52885	+	12
	Intr	53026	53126	+	12
	Term	53219	53342	+	12
	Init	54287	54622	+	13
15	Intr	54674	55422	+	13
	Intr	55674	55739	+	13
	Intr	55827	55971	+	13
	Intr	56061	56279	+	13
	Intr	56374	56572	+	13
20	Intr	56661	56856	+	13
	Term	56946	57063	+	13
	Term	57582	57334	-	14
	Intr	57798	57691	-	14
25	Intr	58158	57874	-	14
	Intr	58526	58242	-	14
	Intr	58879	58598	-	14
	Intr	59149	58966	-	14
	Intr	59437	59282	-	14
30	Intr	59792	59503	-	14
	Init	60069	59872	-	14
	Term	60874	60653	-	15
	Intr	61357	60950	-	15
35	Intr	61667	61505	-	15
	Intr	62077	61798	-	15
	Intr	62421	62168	-	15
	Init	62629	62503	-	15
40	>5541660 /				
	len =	71220	nex =	95	
	Sngl	846	388	-	0
45	Init	1261	2018	+	1
	Term	2063	2339	+	1
	Term	2992	2955	-	2
50	Intr	3174	3078	-	2
	Intr	3428	3266	-	2
	Intr	3573	3502	-	2
	Intr	3791	3657	-	2
	Intr	4092	3892	-	2
55	Init	4671	4258	-	2
	Term	5794	5711	-	3
	Intr	5928	5878	-	3
	Intr	6091	6013	-	3
60	Intr	6482	6379	-	3
	Intr	6644	6563	-	3

	Intr	6846	6726	-	3
	Intr	7097	6938	-	3
	Intr	7231	7170	-	3
	Init	7764	7329	-	3
5					
	Init	8083	8634	+	4
	Intr	8722	8796	+	4
	Intr	9273	9359	+	4
	Intr	9394	9450	+	4
10					
	Intr	9648	9707	+	4
	Intr	9796	9876	+	4
	Intr	9968	10003	+	4
	Term	10090	10170	+	4
15					
	Init	11387	11607	+	5
	Intr	11990	12202	+	5
	Intr	12474	13163	+	5
	Intr	13195	14661	+	5
	Intr	14784	15536	+	5
20					
	Intr	15832	15986	+	5
	Intr	16190	16590	+	5
	Term	16653	17015	+	5
25					
	Init	24295	24516	+	6
	Intr	24596	24789	+	6
	Term	24862	25270	-	6
30					
	Term	26197	26099	-	7
	Intr	26404	26306	-	7
	Intr	27329	26629	-	7
	Intr	27606	27513	-	7
	Intr	27926	27760	-	7
	Intr	28093	28000	-	7
	Intr	28281	28172	-	7
35					
	Init	28752	28416	-	7
40					
	Init	32354	34619	+	8
	Term	34765	35285	+	8
45					
	Term	37093	36987	-	9
	Intr	37340	37184	-	9
	Init	38662	38342	-	9
50					
	Init	39578	39623	+	10
	Intr	39702	41048	+	10
	Term	41181	41218	+	10
55					
	Term	42494	41993	-	11
	Intr	43351	43047	-	11
	Intr	44342	43449	-	11
	Intr	44765	44474	-	11
	Intr	46025	45145	-	11
	Intr	46704	46477	-	11
	Init	47832	47425	-	11
60					
	Init	49290	49457	+	12
	Intr	49648	49689	+	12
	Intr	49755	49946	+	12
	Intr	50218	50313	+	12
	Intr	50394	50508	+	12
	Intr	51065	51070	-	12

858

	Intr	51105	51192	+	12
	Intr	51645	51853	+	12
	Intr	51925	52040	+	12
	Intr	52158	52307	+	12
5	Intr	52461	52528	+	12
	Intr	52673	52778	+	12
	Intr	52909	53067	+	12
	Intr	53176	53229	+	12
	Intr	53333	53413	+	12
10	Intr	53516	53653	+	12
	Intr	53975	54134	+	12
	Intr	54364	54443	+	12
	Intr	54540	54688	+	12
	Intr	54781	54838	+	12
15	Intr	54931	54978	+	12
	Term	55141	55284	+	12
	Init	55723	55846	-	13
	Intr	55967	56162	+	13
20	Intr	56249	56476	+	13
	Intr	56592	56823	+	13
	Intr	56903	57010	+	13
	Intr	57095	57367	+	13
	Term	57445	57726	+	13
25					
	Init	60489	60741	+	14
	Term	60774	60778	+	14
	Term	62781	62035	-	15
30	Init	63693	62983	-	15
	Sngl	66901	66713	-	16
35	>5541662 /				
	len =	98462	nex =	118	
	Init	108	225	+	0
	Intr	324	434	+	0
40	Intr	606	659	+	0
	Intr	824	1081	+	0
	Intr	1184	1293	+	0
	Intr	1483	1636	+	0
	Intr	1707	2160	+	0
45	Intr	2248	2462	+	0
	Intr	2558	2796	+	0
	Intr	2889	3015	+	0
	Term	3098	3310	+	0
50	Sngl	4862	3510	-	1
	Sngl	5447	5947	+	2
	Term	6581	6282	-	3
55	Intr	7204	6755	-	3
	Init	8154	7357	-	3
	Init	9457	11794	+	4
	Intr	11847	12079	+	4
60	Intr	12162	12233	+	4
	Intr	12305	12457	+	4

5	Intr	12558	12721	+	4
	Intr	12816	12963	+	4
	Intr	13308	13430	+	4
	Intr	13523	13610	+	4
	Term	13688	13884	+	4
10	Term	14473	14138	-	5
	Intr	14673	14547	-	5
	Intr	14902	14708	-	5
	Intr	15349	15012	-	5
	Intr	15542	15430	-	5
15	Init	15678	15627	-	5
	Init	19089	19105	+	6
	Intr	19139	19364	+	6
	Term	20408	20413	+	6
20	Term	25081	24951	-	7
	Intr	25313	25191	-	7
	Init	25427	25319	-	7
	Sngl	27284	27898	+	8
25	Term	28131	28115	-	9
	Intr	28300	28228	-	9
	Intr	28514	28414	-	9
	Intr	28813	28771	-	9
	Init	29021	28905	-	9
30	Term	30130	30075	-	10
	Intr	30681	30303	-	10
	Init	31303	30920	-	10
35	Term	33911	33715	-	11
	Init	34080	34020	-	11
40	Term	35404	34872	-	12
	Intr	35734	35527	-	12
	Intr	35934	35823	-	12
	Intr	36236	36021	-	12
	Intr	36412	36318	-	12
45	Intr	37017	36507	-	12
	Intr	37361	37109	-	12
	Intr	37574	37483	-	12
	Intr	37778	37684	-	12
	Init	37976	37881	-	12
50	Term	39449	39084	-	13
	Intr	40739	40237	-	13
	Intr	41067	40860	-	13
	Intr	41247	41136	-	13
	Intr	41543	41328	-	13
55	Intr	41719	41625	-	13
	Intr	42328	41818	-	13
	Intr	42677	42425	-	13
	Intr	42901	42810	-	13
	Intr	43101	43007	-	13
60	Init	43303	43205	-	13
	Init	43784	43787	+	14
	Intr	44307	44716	+	14

860

	Intr	45919	46116	+	14
	Term	47127	47615	+	14
5	Init	50322	50526	+	15
	Intr	51049	51608	+	15
	Term	51694	51867	+	15
	Sngl	53782	55842	+	16
10	Init	57772	59044	+	17
	Intr	59258	59566	+	17
	Intr	60415	62335	+	17
	Term	62797	62974	+	17
15	Term	63812	63293	-	18
	Intr	64040	63982	-	18
	Intr	64260	64192	-	18
	Intr	64854	64387	-	18
	Intr	65304	65132	-	18
20	Intr	65500	65365	-	18
	Intr	66102	66047	-	18
	Intr	69249	69084	-	18
	Intr	69444	69332	-	18
	Intr	69894	69537	-	18
25	Init	70250	70143	-	18
	Term	72277	71274	-	19
	Intr	73188	72978	-	19
	Intr	73755	73262	-	19
30	Intr	74159	73845	-	19
	Init	74370	74268	-	19
	Init	75018	76208	+	20
	Intr	76487	76708	+	20
35	Intr	76797	76904	+	20
	Intr	77024	77215	+	20
	Intr	77294	77542	+	20
	Intr	77629	77731	+	20
	Intr	77756	77883	+	20
40	Term	77971	78210	+	20
	Sngl	78702	81245	+	21
	Sngl	81966	83384	+	22
45	Sngl	85282	87825	+	23
	Init	90153	90175	+	24
	Term	90657	90984	+	24
50	Intr	92466	92686	-	25
	Intr	92718	92932	+	25
	Term	93008	93885	+	25
55	Init	94553	94571	+	26
	Intr	95083	95598	+	26
	Term	95678	96555	+	26
	Init	97572	98055	+	27
60	Term	98132	98441	+	27

>5541692

/

len = 87240 nex = 116

5	Sngl	987	56	-	0
	Init	2550	2892	+	1
	Intr	3699	4246	+	1
	Intr	4340	4714	+	1
10	Intr	4823	4858	+	1
	Intr	4958	5080	+	1
	Intr	5164	5271	+	1
	Intr	5362	5418	+	1
	Intr	5525	5587	+	1
15	Intr	5670	5911	+	1
	Intr	5989	6070	+	1
	Intr	6550	6721	+	1
	Intr	6860	6987	+	1
	Intr	7090	7232	+	1
20	Term	7436	7466	+	1
	Init	8989	8991	+	2
	Intr	9054	9847	+	2
	Intr	9905	9959	+	2
25	Intr	10165	10608	+	2
	Intr	10866	10972	+	2
	Intr	11253	11361	+	2
	Intr	11619	11738	+	2
	Intr	12009	12248	+	2
30	Intr	12329	12475	+	2
	Intr	12556	12646	+	2
	Intr	12725	12843	+	2
	Intr	12917	13087	+	2
	Term	13187	13234	+	2
35	Term	14074	13828	-	3
	Intr	15512	14193	-	3
	Intr	15766	15546	-	3
	Intr	15941	15891	-	3
40	Intr	16099	16049	-	3
	Init	16477	16181	-	3
	Init	20027	20332	+	4
	Intr	20850	21040	+	4
45	Intr	21121	21256	+	4
	Intr	21353	21669	+	4
	Intr	21770	21887	+	4
	Intr	22066	22215	+	4
	Intr	22282	22365	+	4
50	Intr	22457	22700	+	4
	Term	23295	23470	+	4
	Term	28207	28106	-	5
	Intr	28614	28296	-	5
55	Intr	28761	28697	-	5
	Init	28976	28833	-	5
	Sngl	29573	31780	+	6
60	Init	32880	33059	+	7
	Term	33139	33387	+	7

	Term	34092	33914	-	8
	Intr	34702	34168	-	8
5	Intr	34931	34773	-	8
	Intr	35815	35012	-	8
	Init	36333	36016	-	8
	Term	37787	37557	-	9
	Intr	37988	37881	-	9
10	Intr	38247	38080	-	9
	Intr	38465	38331	-	9
	Intr	38848	38546	-	9
	Intr	39146	38985	-	9
	Intr	39557	39225	-	9
15	Intr	39735	39640	-	9
	Intr	39962	39824	-	9
	Intr	40934	40854	-	9
	Init	43511	43450	-	9
20	Init	46269	46448	+	10
	Intr	46816	47008	+	10
	Intr	47119	47204	+	10
	Intr	47666	47797	+	10
	Intr	48085	48213	+	10
25	Term	48309	48515	+	10
	Init	50533	50715	+	11
	Intr	51061	51253	+	11
	Intr	51380	51465	+	11
30	Intr	51617	51748	+	11
	Intr	51837	51965	+	11
	Term	52058	52264	+	11
	Term	53234	53092	-	12
35	Intr	53480	53367	-	12
	Init	53858	53747	-	12
	Sngl	57216	57824	+	13
40	Init	61515	61871	+	14
	Intr	61974	62108	+	14
	Intr	62185	62284	+	14
	Intr	62360	62485	+	14
	Intr	62576	62889	+	14
45	Intr	62996	63115	+	14
	Intr	63221	63472	+	14
	Intr	63558	63683	-	14
	Term	63816	64076	+	14
50	Init	65050	65800	+	15
	Intr	66188	66523	+	15
	Term	66612	67309	-	15
	Sngl	68881	69216	-	16
55	Term	72539	71962	-	17
	Init	73600	72637	-	17
	Sngl	77944	78042	+	18
60	Init	80219	80406	-	19

863

	Intr	80618	81087	+	19
	Intr	81693	81943	+	19
	Term	82028	82408	+	19
5	Term	82865	82616	-	20
	Intr	83056	82947	-	20
	Intr	83425	83324	-	20
	Intr	83625	83488	-	20
10	Intr	83832	83714	-	20
	Intr	84040	83995	-	20
	Intr	84363	84343	-	20
	Intr	84592	84511	-	20
	Intr	84768	84689	-	20
15	Intr	84928	84842	-	20
	Intr	85190	85005	-	20
	Init	85670	85272	-	20
	Init	86186	86503	+	21
	Term	86592	87207	+	21
20					
	>5541715 /				
	len =	90780	nex =	145	
25	Term	72	1	-	0
	Init	462	159	-	0
	Term	1132	1087	-	1
	Intr	1388	1218	-	1
30	Intr	1597	1475	-	1
	Intr	2163	2041	-	1
	Intr	2356	2268	-	1
	Intr	2754	2622	-	1
	Init	3149	3063	-	1
35	Term	3745	3703	-	2
	Intr	3963	3841	-	2
	Intr	4336	4212	-	2
	Intr	4959	4837	-	2
40	Intr	5150	5062	-	2
	Intr	5388	5286	-	2
	Init	5644	5582	-	2
	Init	10758	11176	+	3
45	Intr	11291	11316	+	3
	Intr	11668	11698	+	3
	Intr	11844	11931	-	3
	Intr	12065	12213	+	3
	Intr	13021	13100	+	3
50	Term	13315	13562	+	3
	Term	14097	13924	-	4
	Init	16987	14453	-	4
55	Init	17210	17291	+	5
	Intr	17368	17833	+	5
	Intr	17968	18085	+	5
	Intr	18232	18341	+	5
	Intr	18427	18512	+	5
60	Intr	18635	18780	+	5
	Term	18898	18951	+	5

	Init	20458	21032	+	6
	Intr	21307	21436	+	6
	Intr	21812	21977	+	6
5	Intr	22063	22181	+	6
	Intr	22269	22349	+	6
	Intr	22527	22613	+	6
	Intr	22732	22806	+	6
	Intr	22896	22964	+	6
10	Intr	23038	23118	+	6
	Intr	23212	23346	+	6
	Intr	23436	23492	+	6
	Term	23602	23766	+	6
15	Init	26506	27657	+	7
	Term	27890	28204	+	7
	Term	31427	31153	-	8
	Intr	31771	31529	-	8
20	Intr	31980	31857	-	8
	Init	32877	32413	-	8
	Init	34620	35807	+	9
	Term	35890	36195	+	9
25	Term	38525	38336	-	10
	Intr	39060	38860	-	10
	Intr	40278	40070	-	10
	Intr	40543	40445	-	10
30	Intr	41749	40849	-	10
	Init	41968	41832	-	10
	Init	42452	42504	+	11
	Intr	42971	43715	+	11
35	Intr	43778	43918	+	11
	Term	44046	44312	+	11
	Sngl	44908	45429	+	12
40	Init	46357	46547	+	13
	Intr	46861	46963	+	13
	Intr	47051	47339	+	13
	Term	47431	47573	+	13
45	Init	49493	49622	+	14
	Intr	49792	49879	+	14
	Intr	49965	50051	+	14
	Term	50148	50201	+	14
50	Term	50889	50756	-	15
	Intr	51149	51021	-	15
	Intr	51385	51242	-	15
	Intr	51658	51533	-	15
	Intr	52044	51919	-	15
55	Intr	52226	52134	-	15
	Intr	52464	52329	-	15
	Intr	52672	52544	-	15
	Intr	52906	52787	-	15
	Intr	53227	53048	-	15
60	Intr	53500	53321	-	15
	Intr	53845	53711	-	15

	Intr	54165	53953	-	15
	Intr	54417	54329	-	15
	Intr	54703	54554	-	15
	Intr	54927	54789	-	15
5	Intr	55248	55027	-	15
	Intr	55557	55372	-	15
	Intr	55769	55647	-	15
	Intr	55965	55865	-	15
	Init	56473	56173	-	15
10	Init	57438	57490	+	16
	Intr	57576	57615	+	16
	Intr	57896	57976	+	16
	Intr	58059	58253	+	16
15	Intr	58358	58468	+	16
	Intr	58579	58644	+	16
	Intr	58716	58823	+	16
	Intr	58928	58972	+	16
	Intr	59263	59319	+	16
20	Intr	59710	59804	+	16
	Intr	60071	60257	+	16
	Intr	60467	60529	+	16
	Intr	60638	60744	+	16
	Intr	60915	61052	+	16
25	Intr	61131	61212	+	16
	Intr	61322	61392	+	16
	Intr	61951	62031	+	16
	Intr	62396	62453	+	16
	Intr	62557	62609	+	16
30	Intr	62718	62811	+	16
	Term	62904	62993	+	16
	Term	64537	63431	-	17
	Intr	65022	64609	-	17
35	Intr	65227	65111	-	17
	Init	65755	65474	-	17
	Term	66538	66497	-	18
	Intr	66875	66789	-	18
40	Intr	67069	66980	-	18
	Intr	67488	67383	-	18
	Intr	67679	67585	-	18
	Intr	67905	67822	-	18
	Intr	68423	68344	-	18
45	Intr	68730	68690	-	18
	Init	68954	68941	-	18
	Sngr	69877	69737	-	19
50	Init	72249	72436	+	20
	Intr	72522	72808	+	20
	Intr	73121	73335	+	20
	Intr	73435	73458	+	20
	Intr	73818	73998	+	20
55	Intr	74076	74297	+	20
	Intr	74390	74525	+	20
	Intr	74768	74949	+	20
	Intr	75101	75207	+	20
	Term	75296	75378	+	20
60	Init	77483	79961	+	21

866

	Term	80157	81979	+	21
	Term	83660	83541	-	22
5	Intr	83890	83750	-	22
	Intr	84741	84446	-	22
	Init	85162	84859	-	22
	Term	89783	89043	-	23
10	Init	90268	90095	-	23
	>5541718 /				
	len =	103889	nex =	195	
15	Init	252	315	+	0
	Intr	471	526	+	0
	Intr	815	886	+	0
	Intr	989	1071	+	0
	Intr	1185	1260	+	0
20	Intr	1526	1615	+	0
	Intr	1717	1803	+	0
	Intr	1942	2013	+	0
	Intr	2125	2183	+	0
	Intr	2354	2447	+	0
25	Intr	2661	2879	+	0
	Intr	3037	3131	+	0
	Intr	3319	3409	+	0
	Intr	3511	3615	+	0
	Intr	3701	3739	+	0
30	Intr	3840	3929	+	0
	Intr	4080	4181	+	0
	Intr	4230	4502	+	0
	Term	4579	4677	+	0
35	Init	5850	6413	+	1
	Intr	6789	6840	+	1
	Intr	7556	7642	+	1
	Intr	7763	7827	+	1
	Intr	7931	8098	+	1
40	Term	8213	8263	+	1
	Term	8850	8813	-	2
	Intr	9269	9207	-	2
	Init	9758	9572	-	2
45	Init	12184	12210	+	3
	Intr	12368	12456	+	3
	Term	12862	13012	-	3
50	Term	13602	13513	-	4
	Intr	14969	13719	-	4
	Intr	15729	15417	-	4
	Init	16278	16193	-	4
55	Init	17354	17467	+	5
	Intr	17547	17632	+	5
	Intr	17722	17774	+	5
	Intr	17859	17936	+	5
	Intr	18047	18100	+	5
60	Intr	18192	18303	+	5
	Intr	18466	18533	+	5

	Intr	18625	18700	+	5
	Intr	18808	18869	+	5
	Intr	18967	19372	+	5
5	Term	19459	19559	+	5
	Term	20093	20003	-	6
	Intr	20349	20243	-	6
	Intr	20509	20449	-	6
10	Intr	20748	20597	-	6
	Intr	20913	20836	-	6
	Intr	21158	21045	-	6
	Intr	21678	21602	-	6
	Intr	21837	21771	-	6
15	Intr	22021	21956	-	6
	Intr	22258	22135	-	6
	Intr	22467	22346	-	6
	Intr	22771	22508	-	6
	Intr	22931	22851	-	6
20	Intr	23167	23025	-	6
	Init	23631	23523	-	6
	Sngr	24517	24284	-	7
25	Init	26696	26852	+	8
	Term	26928	27058	+	8
	Init	31341	33848	+	9
	Intr	35122	35202	+	9
30	Intr	35576	35684	+	9
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	Intr	36531	36726	+	9
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35	Intr	37093	37229	+	9
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	Intr	46372	46557	+	9
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5	Intr	48656	48842	+	9
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	Intr	50608	50725	+	9
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	Intr	55600	55698	+	9
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	Intr	59741	59849	+	9
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	Intr	61204	61338	+	9
	Intr	61426	61491	+	9
	Intr	61733	61888	+	9
45	Term	61997	62074	+	9
	Term	62673	62419	-	10
	Intr	62994	62789	-	10
	Intr	63434	63209	-	10
50	Intr	64559	63570	-	10
	Init	66101	64899	-	10
	Term	67869	66662	-	11
	Intr	68153	67939	-	11
55	Intr	68343	68288	-	11
	Intr	68711	68421	-	11
	Intr	68988	68819	-	11
	Intr	69226	69084	-	11
	Intr	69465	69321	-	11
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	Intr	70037	69760	-	11

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	Intr	70740	70605	-	11
	Intr	71016	70872	-	11
5	Init	71960	71905	-	11
	Init	72785	72856	+	12
	Intr	73002	73120	+	12
	Intr	73173	73270	+	12
10	Intr	74410	74514	+	12
	Intr	76259	76444	+	12
	Intr	76771	76823	+	12
	Intr	77826	78297	+	12
	Term	78504	78616	+	12
15	Init	79786	79994	+	13
	Term	80110	80146	+	13
	Init	83196	83331	+	14
20	Intr	83500	83565	-	14
	Intr	83712	83858	+	14
	Intr	84026	84139	+	14
	Intr	84233	84322	+	14
	Intr	84431	84553	+	14
25	Term	84672	85024	+	14
	Term	85977	85583	-	15
	Intr	86597	86026	-	15
	Init	87270	87227	-	15
30	Init	89446	89496	-	16
	Intr	89677	89763	+	16
	Intr	89919	90131	+	16
	Intr	90240	90413	+	16
35	Intr	90537	90590	+	16
	Intr	91134	91280	+	16
	Term	91355	91732	+	16
	Init	98284	98541	+	17
40	Intr	98895	99184	-	17
	Term	99570	99972	+	17
	Init	101094	101250	+	18
45	Term	101335	101720	+	18

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which encodes an amino acid sequence exhibiting at least 40% sequence identity to an amino acid sequence encoded by

5 (a) a nucleotide sequence described in Table 1 or a fragment thereof; or
a complement of a nucleotide sequence shown in Table 1 or a fragment thereof.

2. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which exhibits at least 65% sequence identity to

(a) a nucleotide sequence shown in Table 1 or a fragment thereof; or
5 (b) a complement of a nucleotide sequence described in Table 1 or a fragment thereof.

3. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which exhibits at least 65% sequence identity to a gene comprising

(a) a nucleotide sequence shown in Table 1 or a fragment thereof; or
5 (b) a complement of a nucleotide sequence described in Table 1 or a fragment thereof.

4. An isolated nucleic acid molecule which is the reverse of the isolated nucleotide sequence according to claim 1, such that the reverse nucleotide sequence has a sequence order which is the reverse of the sequence order of said isolated nucleotide sequence according to claim 1.

5. An isolated nucleic acid molecule comprising a nucleic acid capable of hybridizing to a nucleic acid having a sequence selected from the group consisting of:

(a) a nucleotide sequence which is shown in Table 1; and
5 (b) a nucleotide sequence which is complementary to a nucleotide sequence shown in Table 1;

under conditions that permit formation of a nucleic acid duplex at a temperature from about 40°C and 48°C below the melting temperature of the nucleic acid duplex.

6. The nucleic acid molecule according to claim 1, wherein said nucleic acid comprises an open reading frame.

7. The isolated nucleic acid molecule of claim 1, wherein said nucleic acid is capable of functioning as a promoter, a 3' end termination sequence, an untranslated region (UTR), or as a regulatory sequence.

8. The isolated nucleic acid molecule of claim 7, wherein said nucleic acid is a promoter and comprises a sequence selected from the group consisting of a TATA box sequence, a CAAT box sequence, a motif of GCAATCG or any transcription-factor binding sequence, and any combination thereof.

9. The isolated nucleic acid molecule of claim 7, wherein the nucleic acid sequence is a regulatory sequence which is capable of promoting seed-specific expression, embryo-specific expression, ovule-specific expression, tapetum-specific expression or root-specific expression of a sequence or any combination thereof.

10. A vector construct comprising a nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is heterologous to any element in said vector construct.

11. A vector construct comprising:

(a) a first nucleic acid having a regulatory sequence capable of causing transcription and/or translation; and

(b) a second nucleic acid having the sequence of the isolated nucleic acid molecule according to claim 1;

wherein said first and second nucleic acids are operably linked and

wherein said second nucleic acid is heterologous to any element in said vector construct.

12. The vector construct according to claim 11, wherein said first nucleic acid is native to said second nucleic acid.

13. The vector construct according to claim 11, wherein said first nucleic acid is heterologous to said second nucleic acid.

14. A vector construct comprising:

(c) a first nucleic acid having the sequence of the isolated nucleic acid molecule according to claim 7; and

(d) a second nucleic acid;

wherein said first and second nucleic acids are operably linked and

wherein said first nucleic acid is heterologous to any element in said vector construct.

15. The vector construct according to claim 14, wherein said first nucleic acid is native to said second nucleic acid.

16. The vector construct according to claim 14, wherein said first nucleic acid is heterologous to said second nucleic acid.

17. A host cell comprising an isolated nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is flanked by exogenous sequence.

18. A host cell comprising a vector construct of claim 10.

19. A host cell comprising a vector construct of claim 11.

20. A host cell comprising a vector construct of claim 12.

21. A host cell comprising a vector construct of claim 13.

22. A host cell comprising a vector construct of claim 14.

23. A host cell comprising a vector construct of claim 15.

24. A host cell comprising a vector construct of claim 16.

25. An isolated polypeptide comprising an amino acid sequence

(a) exhibiting at least 40% sequence identity of an amino acid sequence encoded by a sequence shown in Table 1 or a fragment thereof; and

(b) capable of exhibiting at least one of the biological activities of the polypeptide encoded by said nucleotide sequence shown in Table 1 or a fragment thereof.

26. The isolated polypeptide of claim 25, wherein said amino acid sequence exhibits at least 75% sequence identity to an amino acid sequence encoded by a sequence shown in Table 1 or a fragment thereof.

27. The isolated polypeptide of claim 25, wherein said amino acid sequence exhibits at least 85% sequence identity to an amino acid sequence encoded by a sequence shown in Table 1 or a fragment thereof.

28. The isolated polypeptide of claim 25, wherein said amino acid sequence exhibits at least 90% sequence identity to an amino acid sequence encoded by a sequence shown in Table 1 or a fragment thereof.

29. An antibody capable of binding the isolated polypeptide of claim 25.

30. A method of introducing an isolated nucleic acid into a host cell comprising:

(a) providing an isolated nucleic acid molecule according to claim 1; and

(b) contacting said isolated nucleic with said host cell under conditions that permit insertion of said nucleic acid into said host cell.

31. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 10.

32. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 11.

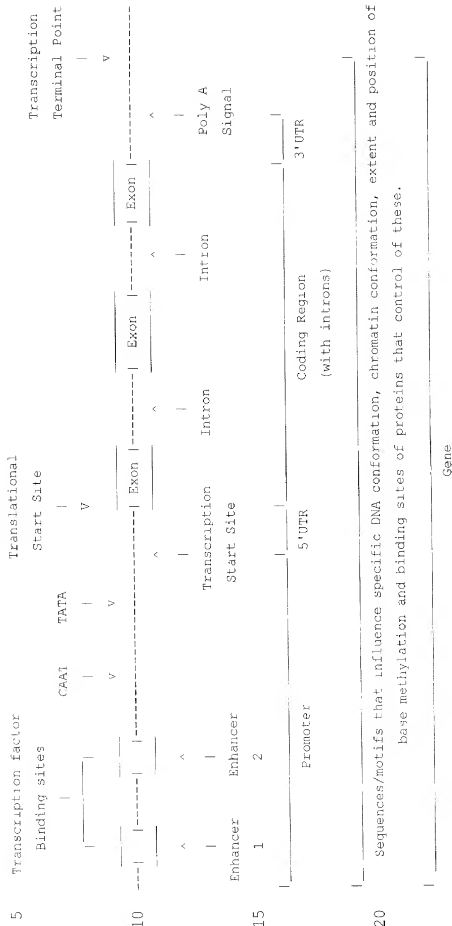
33. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 12.

34. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 13.
35. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 14.
36. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 15.
37. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 16.
38. A method of modulating transcription and/or translation of a nucleic acid in a host cell comprising:
 - (a) providing the host cell of claim 17; and
 - (b) culturing said host cell under conditions that permit transcription or translation.
39. A method for detecting a nucleic acid in a sample which comprises:
 - (a) providing an isolated nucleic acid molecule according to claim 1;
 - (b) contacting said isolated nucleic acid molecule with a sample under conditions which permit a comparison of the sequence of said isolated nucleic acid molecule with the sequence of DNA in said sample; and
 - (c) analyzing the result of said comparison.
40. The method according to claim 39, wherein said isolated nucleic acid molecule and said sample are contacted under conditions which permit the formation of a duplex between complementary nucleic acid sequences.
41. A plant or cell of a plant which comprises a nucleic acid molecule according to claim 1 which is exogenous to said plant or plant cell.
42. A plant or cell of a plant which comprises a nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is heterologous to said plant or said cell of a plant.
43. A plant or cell of a plant which has been transformed with a nucleic acid molecule according to claim 1.
44. A plant or cell of a plant which comprises a vector construct according to claim 10.
45. A plant or cell of a plant which has been transformed with a vector construct according to claim 10.
46. A plant which has been regenerated from a plant cell according to claim 41.

47. A plant which has been regenerated from a plant cell according to claim 42.
48. A plant which has been regenerated from a plant cell according to claim 43.
49. A plant which has been regenerated from a plant cell according to claim 44.
50. A plant which has been regenerated from a plant cell according to claim 45.

SCHEMATIC 1

SCHEMATIC OF A GENE



Sequences/motifs that influence specific DNA conformation, chromatin conformation, extent and position of base methylation and binding sites of proteins that control of these.

ABSTRACT OF THE DISCLOSURE

The present invention provides DNA molecules that constitute fragments of the genome of a plant, and polypeptides encoded thereby. The DNA molecules are useful for specifying a gene product in cells, either as a promoter or as a protein coding sequence or as an UTR or as a 3' termination sequence, and are also useful in controlling the behavior of a gene in the chromosome, in controlling the expression of a gene or as tools for genetic mapping, recognizing or isolating identical or related DNA fragments, or identification of a particular individual organism, or for clustering of a group of organisms with a common trait.

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POWER OF ATTORNEY

CERES, INC.
3007 Malibu Canyon Road
Malibu, CA 90265

I, Richard Hamilton, Chief Financial Officer of CERES, INC. of 3007 Malibu Canyon Road, Malibu, California 90265, grant Power of Attorney and authority to empower the following attorneys to act on behalf of CERES, INC. for executing Verified Statements (Declarations) Claiming Small Entity Status to be submitted to the U.S. Patent and Trademark Office in connection with the filing of provisional or regular patent applications on behalf of CERES, INC.

Raymond C. Stewart (Reg. No. 21,066)
Joseph A. Kolasch (Reg. No. 22,463)
Leonard R. Svensson (Reg. No. 30,330)
Gerald M. Murphy, Jr. (Reg. No. 28,977)
Mark J. Nuell (Reg. No. 36,623)

This Power of Attorney is to remain in full force and effect until terminated by an official of CERES, INC.

By


Richard Hamilton

Date

9/24/98